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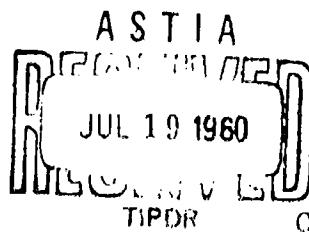
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Technical Report

TOXICITY OF CHEMICALS TO MARINE
BORERS - I



U. S. NAVAL CIVIL ENGINEERING LABORATORY
Port Hueneme, California



TOXICITY OF CHEMICALS TO MARINE BORERS-I

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Interim Report

29 June 1960

by

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U. S. NAVAL CIVIL ENGINEERING
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Port Hueneme, California

407

OBJECT OF TASK

To develop materials and techniques for treating timbers to retard or prevent marine borer attack.

ABSTRACT

The Laboratory has been conducting an investigation of methods for reducing damage to wooden marine structures by marine borers. One phase of the program has been to employ assays of toxicity as laboratory screening tests to determine which substances could be eliminated from consideration and which substances merited further investigation.

Methods for evaluating the toxicity of chemical agents to marine borers were developed and a method was devised for processing the data on a digital computer. Toxicity data covering the testing of several hundred compounds and mixtures were processed. The purpose of this report is to organize, condense, and interpret results obtained at NCEL between July 1954 and January 1959 together with all available data on the toxicity of chemical agents to marine wood-boring organisms.

About a third of the many constituents of creosote tested by the Laboratory were toxic to marine borers. The aliphatic constituents were not toxic either to adult Limnoria or Teredo larvae. With the exception of the xylenols, the monocyclic constituents also were not toxic. The bicyclic compounds and all constituents of creosote that contain two and only two benzene nuclei (such as naphthalene, diphenyl, dibenzyl, diphenylene oxide, and acridine) were toxic to both species of test animals.

With the exception of diphenylene compounds containing two benzene nuclei and a third nonbenzenoid nuclei, polycyclic constituents containing more than two cyclic nuclei were not toxic to Limnoria. Some of them were very toxic to Teredo larvae, however. These polycyclic compounds are the least soluble and least volatile of the many constituents of creosote.

Chlorinated hydrocarbon insecticides (especially endrin and lindane), parathion, and N-methyl-1-naphthyl carbamate were extremely toxic to adult Limnoria but not to Teredo larvae. On the other hand, many pesticides were considerably less toxic to marine borers than they are to other organisms. These included pentachlorophenol, benzyl benzoate, dichlorophene, hexachlorophene, and fluoroacetic acid. Other haloacetic acids and their esters were very toxic to both species of borers.

Of the inorganic and organo-metallic compounds, those containing mercury were the most toxic. Tributyltin compounds were also highly toxic. Copper compounds were very toxic, but were difficult to assay because they reacted with sea water to form insoluble precipitates.

A number of compounds possessing positively charged quaternary or tertiary nitrogen atoms were toxic to Teredo larvae in very low concentrations, but not to adult Limnoria. These included certain of the surface-active agents, several basic organic dyes, and alkaloids extracted from greenheart. Compounds containing negatively charged sulfonic acid groups were, in general, not toxic to either species of test animals.

The results of the toxicity tests have been and are being employed as a guide in the selection of compounds for harbor testing. It is recommended that the toxicity testing program be continued and that the effectiveness of the toxic compounds as wood preservatives be tested in the harbor and that no further consideration be given the compounds which were not toxic. The compounds should be tested individually and as mixtures.

It is planned that in future testing emphasis will be placed upon determining the relationship between chemical structure and the toxicity of compounds containing two benzene nuclei. Attempts will be made to determine their mechanism of action. Further investigations of newer pesticides, organo-tin compounds, and compounds possessing positively charged nitrogen atoms will also be emphasized.

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INTRODUCTION

The mere fact that a chemical is black, smelly, greasy, sticky, or gummy does not make it a wood preservative for marine structures. It must also be toxic. In tests performed at this laboratory silicon oil, petroleum oil, carbon black, paraffin, petrolatum, and distillation residues of creosote were all unable to protect matchsticks from Limnoria. Equally ineffective were gummy materials such as the polyvinyl acetate copolymers (bubble gum), or odiferous materials such as the alkyl mercaptans. Even hard, tough films of polyester casting resin did not deter the borers. Since these nontoxic materials cannot protect small pieces of wood from marine borers for more than a few days in the laboratory, they cannot be expected to protect piling for years in the ocean.

The principal reason for the success of creosote as a preservative of marine piling is that it is very toxic to marine borers and possibly to other marine organisms. Some of the other chemicals that have been used to preserve marine piling are more toxic per unit weight, but these need to be diluted with a solvent carrier before they can be used to treat wood. Creosote, on the other hand, being a liquid, needs no solvent and may be stored undiluted within the hollow sapwood cells of certain softwood timbers. With no other presently employed preservative is it possible to store within wood a greater capacity to kill marine borers, and none cost less than creosote per unit killing power.

An experiment by the Laboratory which clearly illustrates the importance of toxicity has been described.^{1,2} It was shown that creosote extracted from the surface layer of an area of piling honeycombed by the burrows of Limnoria was only slightly toxic to them, whereas creosote extracted from the same piling from an area not invaded by the borers was highly toxic to them.

Hypothetically, wood preservatives could function by virtue of properties or qualities other than toxicity. For example, because of its toughness or hardness, a preservative might physically prevent a borer from chewing into the wood. Indeed, protective coatings of various types, ranging from concrete encasements to metal sheathing, are effective in preventing marine borer attack. No doubt protective coatings of any of a number of synthetic materials would be effective. However, as has been determined in experiments performed at this laboratory, the very thin films that coat individual fibers of wood impregnated with dilute solutions of resinous material do not prevent borer attack.

Agents that react chemically with wood, converting it into new materials that cannot be digested by the borers, might also function to preserve marine timbers. No such agent has been found. Furthermore, conversion of wood into new materials is a field of investigation in itself and chemicals used in this conversion are wood preservatives only in the broadest sense of the term.

An agent that is not toxic to marine borers but that is nevertheless repulsive to them because of its odor or taste might also function as a preservative. No such agent has been demonstrated to exist, however, and methods have not been developed to test for repellency to marine borers. In fact, it has not been established that marine borers have a sense of smell or taste.

Only chemicals toxic to marine borers have been proven to be effective in preserving marine timbers, but factors other than toxicity, such as volatility, solubility in sea water, and affinity for wood fibers, are also of prime importance. Thus it is unlikely that the ratings assigned to a group of poisonous compounds in a toxicity test would resemble the ratings assigned to the same compounds in a test of their effectiveness in protecting wood from marine borer attack.

Though the toxicity of a wood preservative is not the only factor which determines its effectiveness, it is a necessary attribute. Assays of toxicity can, therefore, serve as laboratory screening tests. Through them it is possible to eliminate from consideration those compounds which are not toxic and select for further consideration and testing those which are. Because toxicity tests are relatively inexpensive and convenient and because such a small proportion of candidate materials actually prove to be sufficiently toxic to merit further investigation, such tests are an effective device for screening potential protective materials.

HISTORICAL BACKGROUND

Many investigators have studied the toxicity of chemical agents to wood-destroying organisms, but the majority have been concerned only with wood-destroying fungi. In general, those who have studied the toxicity of chemical agents to marine borers have considered "rapid kill" rather than "low concentration" to be the criteria of high potency. Thus, the exposure periods have usually been brief and many very potent poisons, whose toxic effects are characterized by a slow onset or lag period, have been missed. Because of their fast action, many others have been overrated.

Tests in which exposed adult Bankia have been used as test animals are of dubious value. Out of their natural encasements in the wood, these borers cannot maintain sufficient internal hydrostatic pressures to perform their necessary life functions. Tests performed with Bankia in their natural burrows in the wood are complicated by the ability of these borers to seal their burrows from an unfavorable environment.

Quatrefages³ (1854) used spermatazoa of teredine borers as test animals. Corrosive sublimate in a dilution of 1:2,000,000 killed the spermatazoa in 40 minutes; a dilution of 1:20,000,000 killed them in 2 hours.

Shackell⁴ (1915) studied the effect of various constituents of creosote on the molluscan borer Xylotrya gouldi (Bankia). His test animals were carefully dissected from pine boards which had been exposed to the attacks of the borers for not more than five months. The test consisted of immersing the animals in sea water suspensions of the test agent for periods of 10, 20, 30, 40, 50, and 60 minutes. Six specimens were employed for each chemical agent tested. The animals were then transferred to sea water containing no added chemicals and the times of their deaths were noted. Gum arabic in a concentration of 400 ppm (parts per million) was employed to disperse the agents in sea water. Filtered saturated solutions of the agents were also tested.

The materials tested by Shackell were various distillation fractions of creosote and various compounds which are constituents of creosote. Shackell concluded that the toxicity of creosote fractions diminish with rise of boiling point, the toxicity of creosote itself lying between that of the lowest and highest boiling fractions. He concluded that naphthalene and anthracene are practically nontoxic for Xylotrya, whereas the tar acids, especially alpha-naphthol, are very toxic.

In a later investigation (1916), Shackell⁵ studied the effects of the same agents upon adult Limnoria and came to the same conclusions. He did not describe the method employed for the Limnoria tests but merely stated that the methods were very similar to those used for the tests with Xylotrya. One would conclude that the exposure periods were very brief.

In a final paper (1923), Shackell⁶ gathered information on the survival and recovery of Limnoria first exposed to sea water solutions of various poisons and then transferred to untreated sea water. These experiments were reviewed (1955) by Clapp and Kenk⁷ and the following statements were obtained from that review:

"Limnoria lignorum was exposed to various concentrations of phenol; ortho-, meta-, and para-cresol; pyrocatechin; resorcinol; hydroquinone; or pyrogallol.

The time required to induce complete paralysis of the animals, that required for recovery, and the relation between duration of paralysis and number of animals recovering were recorded. The velocity of poisoning by phenols was nearly proportional to the square of their concentration."

Yonge and Barger⁸ (1922) used Bankia larvae as test animals. The larvae were exposed to several concentrations of each agent tested, each concentration being half that of the next higher in the series. At the end of 24 hours the larvae were examined and for each compound the minimum concentration to kill 100 percent of the larvae was determined.

Yonge gave the following values for the minimum lethal concentrations: corrosive sublimate, 0.0003125 grams per 100 cubic centimeters; copper chloride, 0.0125; tartar emetic, 0.00625; calomel, 0.015; zinc sulphate, 0.00375; and arsenious acid, 0.000875. Saturated solutions of stannous chloride, lead nitrate, and sodium fluoride were ineffective.

An insoluble organic arsenic compound, D. M. (adamsite or chlorodihydro-phenarsazine), killed Bankia larvae when added to the water in powdered form even in very minute quantities. The element arsenic was ten times as effective when used as D. M. as when used as arsenious acid.

Allen and Carter⁹ (1924) employed four different methods for evaluating toxicity: Limnoria tests, tests on Bankia encased in their natural burrows in the wood, tests on Bankia removed from their burrows, and tests on Bankia embryos. The same concentrations, namely 10 and 20 ppm, were used for all compounds and methods tested.

In the Limnoria tests, 20 adult animals were employed for each concentration of each compound tested. Throughout the test, which was run for 120 hours, the animals were immersed in the test solutions. The criteria of toxicity was the time required to kill 90 to 100 percent of the animals.

The tests employing exposed Bankia were performed in a similar manner. Three specimens in 150 milliliters of test solution were used for each of the two concentrations of each compound tested. Tests on Bankia in wooden blocks were performed by immersing the entire block in the test solutions for periods up to seven days. The blocks were then transferred to sea water containing no added chemicals and, after a period of waiting, counts were made of the number of retractable siphons extruding from the wood. As similar counts had been made before the immersion in the toxic solutions, the percent of animals killed could be calculated. A single concentration schedule of 20 ppm was employed for all compounds. The criteria for toxicity in the Bankia embryo test was minutes to cause a 100-percent kill.

Allen and Carter concluded that the toxicity of the various compounds is approximately the same for Limnoria, exposed Bankia, and Bankia embryos. However, they concluded that Bankia encased in their natural burrows in the wood are more resistant to the toxic agents. Of the 45 compounds tested, the following compounds had the greatest toxicity values in order of descending toxicity:

1. Chlorovinyl arsenious oxide	7. Cupric α -nitrobenzoate
2. Phenyl arsenious oxide	8. Cuprous chloride
3. Mercuric oxide	9. Mercuric anilinate
4. Mercuric chloride	10. Mercuric benzoate
5. Mercuric arsenate	11. Crystal violet
6. Cuprous cyanide	

These 11 compounds were selected by Allen and Carter because they were able to cause deaths in a short period of time. The same concentrations of other compounds, such as Paris green and benzalilide, also killed all of the species of test animals but were not classified as highly toxic because they did not kill the animals quickly.

White¹⁰ (1929), using Limnoria lignorum as test animals, tested the toxicity of about 80 compounds. Vigorous adult Limnoria were exposed to various dilutions of the test compounds for 18 hours, at which time observations were made of the number dead. A single animal was employed in each dish, with two dishes employed for each concentration of each compound tested. The highest concentration employed was 20 ppm. Very insoluble compounds were tested as suspensions at a concentration of 10 ppm.

The substances which were found to be toxic together with their minimum lethal concentrations are listed below:

1. Fluorenone (2 ppm)
2. Mercuric chloride (5 ppm)
3. Acridine (7 ppm)
4. Diphenylchlorarsine (10 ppm)
5. 2-Phenanthrol (10 ppm)
6. Fluorenyl alcohol (10 ppm)
7. Diphenyl iodoarsine (20 ppm)
8. Diphenyl arsenious oxide (20 ppm)
9. Sodium arsenite (20 ppm)
10. 6-Chlorophenoxyarsine (20 ppm)
11. Alpha-Naphthol (20 ppm)
12. Beta-Naphthol (20 ppm)
13. Nitronaphthalene (20 ppm)

Mayfield¹¹ (1951) reviewed the literature pertaining to the toxicity of various fractions of creosote to fungi, insects, and marine borers. His paper included a detailed discussion of the development of methods for determining toxicity, but the discussion was concerned principally with tests on wood-destroying fungi.

Lane¹² (1951) devised a microtechnique for studying the effectiveness of sublethal concentrations of toxic substances on the rate of oxygen consumption of *Teredo* larvae. The apparatus he employed, a microrespirometer, consisted of a 0.5-millimeter capillary glass tube with a pear-shaped respiration chamber blown on one end and a microbarometer attached to the other.

Measurements of the oxygen uptake of *Teredo* larvae immersed in solutions of various agents in sea water were made over periods of about 1-1/2 hours. A concentration of 0.5×10^{-9} grams per milliliter of creosote in sea water reduced the rate of oxygen consumption approximately 50 percent, and a concentration of 0.5×10^{-6} grams per milliliter killed the larvae.

In a very ambitious testing program (1955), Trussel, et al,¹³ studied the ability of over a hundred chemicals to kill adult *Bankia* in their natural burrows in the wood. They were seeking an agent suitable for poisoning the water surrounding timbers which had already become infested with borers. The process they envisioned might be compared to fumigation.

As a screening process for selecting such agents, infested blocks were immersed in test solutions of various compounds for 18 hours. During submersion the siphons of the *Bankia* were observed, withdrawal of the siphons indicating sensitivity to the toxicant. After exposure, the blocks were returned to the ocean for several days. They were then brought back into the laboratory, placed in a jar of sea water for 1/2 hour and then examined for siphon activity. The results were recorded on the following basis: no active siphons - complete kill; one or two active siphons - partial kill; more than two active siphons - no kill.

Most of the one hundred or more compounds tested were toxic but did not enter the wood in sufficient quantity to cause death because the *Bankia* quickly withdrew their siphons and sealed the openings with their pallets. Sodium arsenite was an exception. Even high concentrations of this compound either were not detected by the *Bankia* or caused paralysis of their retractor muscles. Because it could freely enter the burrows of the *Bankia* and was actively pumped through the siphons and into the main body of the animals, sodium arsenite was the most effective of the compounds tested.

Six other of the compounds tested by Trussel, et al, killed all of the borers, even though they did cause the Bankia to retract their siphons during immersion. These were:

1. Capryldinitrophenyl acetate
2. Octadecylmethylammonium salicylate
3. Ethyl pyridine p-chlorbenzenesulphonate
4. S-*t*-octyl-cresoxyethyl-N, N'-dimethylthiouronium chloride
5. Trichlormethyl-p-chlorbenzenesulphonate
6. Isobutyl benzoyl acrylate

Another group of seven compounds, which caused the Bankia to withdraw their siphons during the immersion period, killed most of the borers. These were:

1. N-(Trichloromethylthio)-4-cyclohexene-1, 2 dicarboximide
2. S-Benzyl thiouronium dinitrocaprylphenate
3. Dinitro-*o*-cyclohexylphenol
4. Triethanolamine dinitro-*o*-sec-butylphenate
5. 3-(p-Chlorophenyl)-5-methylrhodanine
6. Sodium *o*-benzylchlorophenate

In a recent paper (1959), Rice and Ballard¹⁴ described a screening test for selecting agents toxic to Limnoria tripunctata. They did not present a list of the compounds tested, however, or any of their testing results. In their procedure, five animals were placed in each of a series of small containers holding 10 milliliters of sea water. Ten milligrams of a test compound, in a suitable solvent, were applied to a small filter paper in a petri dish. After the solvent had evaporated, the test specimens and sea water were introduced. Results were observed 1, 2, and 4 hours later. Compounds demonstrating 100-percent mortality in 1 hour were considered candidates for further testing.

Only the most toxic of the many compounds tested by the various investigators have been listed in this chapter. All the compounds tested by them and the compounds tested at NCEL are itemized in the general index which constitutes Appendix C. This index is believed to list all compounds and chemicals that have been tested on marine borers and reported in the literature.

STATEMENT OF THE PROBLEM

This laboratory has been engaged in testing the toxicity of various chemicals to marine wood-boring organisms since 1954. A major portion of the effort has been devoted to the development of satisfactory procedures for evaluating toxicity. As a result of this program a large amount of data, which covers the testing of several hundred compounds, has been collected. By merely inspecting the data or averaging them roughly, it has been possible to select the most toxic compounds with sufficient accuracy for many purposes. However, the data have not been analyzed critically nor have they been recorded in a form sufficiently condensed to provide a useful and convenient table of toxicities.

The task of organizing, condensing, and interpreting these data is the object of this report. The primary objective is to classify the compounds assayed at this laboratory, with respect to their toxicity to marine borers. A secondary objective is to design a better procedure for evaluating toxicity.

DEVELOPMENT OF THE TESTING PROCEDURES

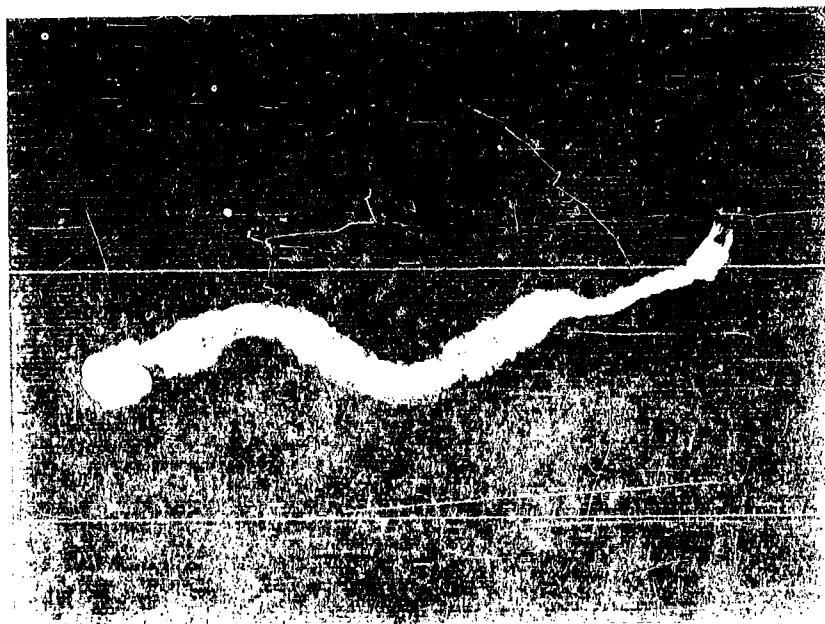
General Testing Procedures

Although changes have been made from time to time in details of the testing procedures, the general plan employed at this laboratory for evaluating the toxicity of chemicals to marine borers has remained unchanged. A number of the borers are placed in petri dishes containing sea water to which various quantities of a chemical agent have been added. After specified intervals, observations are made of the percent of the animals killed. During the test, the animals are immersed in the test solutions or suspensions continuously.

Test Animals

The test animals used in the toxicity testing program are adult Limnoria and larvae of Teredo diegensis. The first of these, a small crustacean, burrows or chews on or near the surface of wood exposed to a marine environment. The molluscan borer Teredo, on the other hand, burrows into the interior of the wood. Whereas adult Limnoria, such as those shown in Figure 1, are no larger than a grain of rice, Teredo (Figure 2) typically grow to a length of from 3 to 6 inches and a diameter of from 1/8 to 1/4 of an inch. Their clamlike larvae, measuring a few tenths of a millimeter in diameter, are expelled into the surrounding water through one of a pair of siphons which extend from the adult.





Teredo larvae are obtained from untreated blocks of southern yellow pine that have become infested with adult Teredo after 8 or 9 months' exposure. The infested collecting blocks are removed from the harbor and placed in an aquarium through which a stream of sea water continually flows. Overflow of water from the aquarium passes over a fine screen upon which the minute larvae are collected (Figure 3).

Before June 1955, Limnoria were also obtained from untreated test blocks of pine or fir which had been exposed in the harbor for 8 or 9 months. The crustacean borers obtained from this source included Limnoria tripunctata, Limnoria quadripunctata, and Chelura. The blocks also harbored many Bankia and Teredo and were well riddled by their tunnels. Furthermore, the surfaces of the blocks were heavily coated with fouling organisms. For these reasons, and because the population of crustacean borers was not great on these test blocks, picking or removing these animals (Figure 4) was a slow and tedious process.

Shortly before June 1955,^{1,2} an old wharf at Port Hueneme was rebuilt and many creosoted timbers that had been in the harbor for about thirteen years were removed. Many of the piles harbored large populations of Limnoria. Eight-foot sections were cut from several of them at the intertidal zone and reserved as a new source of test animals for the toxicity tests.

In confirmation of an earlier report (1951) by Menzies,¹⁵ only a single species of marine borers, Limnoria tripunctata (Menzies), was found. No teredine borers were present and the fouling was very light. From chips a few inches square cut from the surface of these piling sections, it was possible to collect several hundred Limnoria tripunctata in a few hours. Since that time the only species of Limnoria employed in the toxicity tests has been Limnoria tripunctata (Menzies).

Death Among Control Animals

The cause of all of the deaths of marine borers immersed in sea water solutions of a test chemical cannot be ascribed to the presence of the chemical. To calculate the death rate attributable to the test chemical, corrections for the death rate in control dishes containing only sea water must be applied. Assays in which the death rates in the control dishes are high are not very reliable.

Initially, the mortality among the control dishes was quite high in both the Limnoria tests and Teredo larvae tests. One reason for this was that the temperature fluctuated over a wider range than the animals could tolerate and temperature-regulating equipment was therefore installed.



Figure 3. Apparatus for collecting Teredo larvae.



Figure 4. Teasing adult Limnoria from their burrows.

When Limnoria are picked or dug from the wood a number of them are injured and the death rate during the several days following is high. This was the chief cause of deaths in the control dishes during the first year that the Limnoria tests were performed at this laboratory.

For the past several years the Limnoria have been collected well in advance of the test and stored in dishes of clean sea water well supplied with toothpicks. The healthy animals soon attach themselves to the toothpicks and can easily be separated from the dead or injured animals.

Since this procedure has been adopted, deaths among the control animals have been reduced to negligible numbers. This fact is illustrated by the following summary of data obtained for deaths among control animals in the Limnoria tests between the dates of 6 June 1956 and 17 June 1957:

Total number of animals tested	720
Percent dead in 6-1/4 hours	0%
Percent dead in 25 hours	0.42%
Percent dead in 48 hours	0.97%
Percent dead in 72 hours	1.39%
Percent dead in 100 hours	2.22%

The natural mortality of Teredo larvae is quite high. At the end of the customary three-day test period there are quite a number of dead larvae even in the control dishes and often at the end of a week's time half of them are dead. However, there are invariably a few free-swimming larvae still quite active in the control dishes after one week's time and quite a number of others which are alive and active though not free-swimming.

Scrambling System

Until recently the solutions prepared for the toxicity tests had been arranged in sequence and observations of the percent kill had been made in the same order. The observer had been aware of the name of the chemical and the concentration of the solution to which the animals under observation were being exposed. His personal opinion had no doubt frequently influenced the scores assigned.

In 1958 a scrambling system was devised. The dishes of test animals in test solutions were assigned arabic numbers in random order by means of a well-shuffled deck of numbered cards. A record was made of the number assigned to each solution and then the old markings, indicating the test chemical and concentration, were removed. The dishes were then arranged in order according to the new set of random numbers. Thus, the sequence of the compound and the concentration was scrambled and the observer no longer knew the content of the dish he was inspecting.

At present the scrambling system is used in such a manner that it not only arranges the sequence of the observations in a random order but also insures that the different dishes of test animals are assigned to the solutions in random order. This is described in detail in the Standard Procedure, Appendix A.

Quantal and Semiquantal Responses

The response in biological assays is generally assumed to be either quantal (all or none) or graded, but responses often occur which are of an intermediate nature. These have been referred to, e.g. by Finney¹⁶ (1952), as semiquantal. In the marine borer tests, death is a stepwise process and can be looked upon as a semiquantal response.

In much of the early testing at this laboratory, attempts were made to describe the state of health of animals which were in various stages of the death process. Such classifications as dead, moribund, almost dead, immobile, affected, slightly affected, sluggish, and active were used.

Later, numerical systems were adapted. In the Limnoria tests an animal which was no longer able to bore on wood but which was still able to swim about when prodded was classified as 25 percent dead. When an animal could no longer remain upright but fell on its back, actively moving all of its appendages, it was classified as 50 percent dead. When it lay on its back, motionless but for the beating of its pleopods, it was classified as 75 percent dead, and when all motion ceased, 100 percent dead.

As Teredo larvae were too numerous and active to be examined individually, only a single score could be given for each dish. At first the larvae in each beaker at each reading were classified as being active, sluggish, immobile, or dead. Later, a numerical scale of ten was used to describe their state of activity. If the larvae were as active as those in control dishes containing only sea water, they were assigned a score of zero. If all of the larvae were dead, a score of ten was assigned. For intermediate degrees of liveliness, intermediate numbers were assigned.

It was found, however, that agreement of scores obtained by two observers were no better on the semiquantal scales than on a simple "dead" or "not dead" scale. Because the semiquantal scales were complicated and subjective, their use was discontinued.

In the Teredo larvae tests a plus score is now assigned if all of the larvae are dead and a negative score if they are not all dead. In spite of this simplification a certain amount of subjectiveness or guesswork still enters in, as often the larvae close their shells when exposed to toxic solutions and appear to be dead, only to become active again if transferred to untreated sea water.

Duplicate Readings

It is often difficult to decide whether an animal should be classified as "dead" or "not dead" and repeat readings on the same dish often vary. For some time, in both the Limnoria tests and the Teredo larvae tests, duplicate readings have been made. Two independent observers assign scores to the dishes of test animals, and the two scores are averaged.

Choice of Time for Measuring Response

During the several years that the toxicity tests have been performed, a number of questions have been present. Over how long a period should the test run? Is it best to compare the compounds on the basis of the relative concentrations required to kill say half of the animals in 24 hours, in 48 hours, or in 100 hours? Should the animals be exposed to the toxic agent for a stated period of time and then be returned to untreated sea water, the response being noted some time later; or should the animals be exposed continuously to the test solutions? Arbitrary answers to these questions were not found to be satisfactory, as the relative potencies of different compounds depended upon the choice of the time intervals involved.

Duration of Exposure and Survival Time

Two time measurements are inherent in biological experiments in which a response to an agent or stimulus is measured; i.e., the duration of the exposure to the agent or stimulus and the survival time. The difference between these two measurements can be illustrated by a description of experiments in which the lethality of gamma radiation to insects is measured. The insects are exposed to various (high) intensities of gamma radiation for definite (short) periods of time. The durations of these exposure times are sufficiently brief that none of the test animals die during the exposure. The exposures are discontinued and the times required for the animals to die (the survival times) are then measured. Rather than actually measuring the survival time of each animal, usually counts are made periodically of the numbers of animals dead and surviving. The proportion of animals having survival times equal to or less than the time in question is thus determined.

The toxicity of an agent to marine borers would ideally be determined by assays in which the durations of exposure to the test agent and the survival times of the test animals could both be measured. The test animals would be exposed to various (high) concentrations of the agent in sea water. After specified (short) periods of time they would be transferred to sea water containing no added chemicals. At intervals thereafter, counts would be made of the number of animals dead and

surviving. Eventually no further deaths would occur and the total fractions of animals killed by the various exposures to the agent could be estimated. However, one could not be assured that the estimates obtained in this hypothetical procedure were in truth measurements of what they were presumed to be.

Some chemicals enter the body of test animals, such as Limnoria, quite rapidly. The concentration of the chemical within the animal's body quickly reaches equilibrium with the surrounding solution. With other chemicals, equilibrium with the surrounding solution is reached very slowly. A similar situation occurs when the animals are transferred from the test solution to untreated sea water. Some chemicals rapidly diffuse from the animals and other chemicals are retained tenaciously by the tissues of the animals.

Dinitrophenol, a yellow poison, is quickly taken up by Limnoria, and the animals rapidly assume a yellow color. A long period of waiting for the chemical to enter the animal's body is not required and deaths from high concentrations occur in a short period of time. If the animals are then transferred to untreated sea water, they rapidly lose their yellow color and those which were obviously ill or even dying from the effects of dinitrophenol quickly recover. No further deaths occur.

A different situation occurs with compounds such as the toxic mercurated dyes. As with dinitrophenol, the animals are quickly colored and deaths occur rapidly in concentrated solutions. However, the animals do not quickly lose their color when transferred to untreated sea water. Many animals which do not appear to be ill at the time of transfer die a day or two later. It is not justifiable to consider that the duration of the exposure to the toxic dye is limited to the period of immersion in the test solution.

The chlorinated hydrocarbon insecticides present yet another picture. Animals exposed to certain of these poisons rapidly lose their ability to coordinate movement, but they do not die quickly even in high concentrations. However, animals exposed even to extremely low concentrations of some of these pesticides die a hundred or so hours later. This is true even if the animals are exposed to the toxic solutions only for a short time and are then transferred to untreated sea water. Deaths among the animals transferred to untreated sea water are nearly as frequent as those exposed continuously in the toxic solutions.

The death rate of Limnoria exposed to suspensions of creosote is somewhat intermediate between that of those exposed to dinitrophenol and an insecticide of the chlorinated hydrocarbon type, such as endrin. Deaths from high concentrations of creosote do not occur as rapidly as from high concentrations of dinitrophenol, but

the period of waiting before deaths occur is only about one-third as great as the length of the lag period associated with some of the chlorinated hydrocarbon insecticides. Among Limnoria first exposed to suspensions of creosote in sea water and then transferred to untreated sea water are a few that die a day or so later, but the majority recover from the toxic effects of the creosote.

Purpose of Test in Relation to Choice of Time Intervals

The toxicity of sea water solutions of a chemical agent to Limnoria is thus seen to be relative. It depends upon the nature of the exposure and the duration of the test. Choice of the best procedure for measuring toxicity depends upon the purpose for which the test is being performed or the type of agent being sought.

If the assay procedure called for a short period of exposure followed by transfer to untreated sea water, with response being noted sometime later, dinitrophenol would be essentially nontoxic and creosote only slightly toxic, whereas some of the chlorinated hydrocarbon insecticides and the mercurated dyes would be highly toxic. Such a procedure would be suitable for selecting agents to exterminate Limnoria in an entire area of a harbor by periodically dumping chemicals into the area, a process which might be compared to fumigation.

If the procedure called for continuous exposure to the toxic solutions with response noted at 24 hours, creosote would again be rated as slightly toxic and the mercurated dyes as highly toxic. However, dinitrophenol would be rated high and the chlorinated hydrocarbon insecticides would not be toxic. The test would be suitable as a means of selecting an agent which is capable of quickly killing Limnoria with which it comes into contact.

If the assay procedure were based upon the cumulative kill after prolonged continuous exposure, all of these compounds would be highly toxic. This procedure should be well suited for screening potential wood preservatives because animals boring on treated wood are continuously exposed to the preservative as long as they remain on the wood. Whether the animals are killed by the preservative in one day or ten makes little difference. What is of concern is the number of Limnoria a given amount of preservative can exterminate. It is the agent which can kill the greatest number of Limnoria per unit weight that is being sought, not the agent which can kill in the shortest period of time.

Cumulative Response to Prolonged Continuous Exposure

In the testing procedure used at this laboratory, the animals are not transferred to untreated sea water but are immersed in the test solutions throughout

the assay and observations of the number of animals dead are made at several time intervals. These observations are not measures of the response for a given duration of exposure nor are they measures of the survival time of animals exposed to a given dose of test compounds. The values are simply measures of the cumulative response to continuous exposure to the test solutions.

Such an experimental design has practical advantages. The continuous exposure procedure is simple and transfer of animals during the test, a process in which test animals are frequently injured, is unnecessary. Several observations may be made upon each dish of animals and thus more information can be obtained from a given number of specimens.

The final results in the Limnoria tests are calculated in terms of prolonged continuous exposure. With creosote and most of the earlier compounds tested, the kill at 100 hours was nearly as great as the kill at 200 hours. However, with many of the pesticides which have been tested more recently, there is a very marked increase in the number of deaths at 200 hours over that at 100 hours. Therefore, the tests are currently being carried out for 200 hours and in the future the final results will be calculated on the basis of that time.

Continuous exposure assays are the only assays which can be employed with Teredo larvae as it is not feasible to drain off the solutions and transfer the tiny larvae to a new portion of sea water. However, prolonged tests are more difficult to perform because Teredo exist in the free-swimming larval stage for only a limited period of time. Also, they are too small and too active to permit accurate counts of the total number dead and alive. In the Teredo larvae tests, therefore, scores of plus or minus are assigned at the end of three days of continuous exposure, a plus score indicating that all of the larvae are dead and a minus indicating that they are not all dead.

Preliminary results of experiments now under way indicate that it will be possible to lengthen the periods of exposure in the Teredo larvae tests by a few days. Compounds such as fluoranthene, which were not toxic according to the three-day test, are toxic in fairly low concentrations in tests of one-week duration.

Standardization of Procedures

A major difficulty encountered in attempting to reduce the toxicity data that was gathered at this laboratory arose because the testing procedures had not been standardized. Such schedules as the number of animals employed in a given test, the concentrations employed to test each compound, and the intervals elapsing between each observation had not remained constant. This lack of uniformity in the

testing procedure made comparisons of the toxicities of different compounds less accurate and more time-consuming than is now possible. The variables involved in the Limnoria test are now sufficiently well understood to permit the setting up of rigid schedules with reasonable confidence that further changes will not be required.

It was stated in the first part of this report that one of its purposes was to develop better procedures for evaluating toxicity. In partial fulfillment of this objective, a set of STANDARD PROCEDURES for evaluating the toxicity of chemical agents to Limnoria has been prepared. The procedures, which eliminate many of the situations causing difficulty in the past, are described in Appendix A.

A satisfactory set of standard procedures for evaluating the toxicity of chemical agents to Teredo larvae cannot be drawn up until the variables in the test are better understood. Since the toxicity tests with Teredo larvae were first run, the procedure has remained essentially unchanged. Ten-milliliter aliquots of the test solutions are pipetted into 15-milliliter beakers. A drop or two of a suspension of Teredo larvae is then added to each beaker and finally the beakers are covered with a glass plate. On the first, second and third days following, the larvae are examined at a magnification of 20 times.

DATA REDUCTION

Monomially Spaced Data

The first ratings of toxicity in the Limnoria tests were made by comparing the concentrations required to kill half of the animals in a given period of time. The observations of the percent kill were made periodically, usually at the end of 24, 48, and 72 hours; but the ratings of toxicity were based upon a single reading only. The relative toxicities of the test chemicals varied considerably from one time period to another and the ratings so assigned were thus not very satisfactory.

Expressing Toxicity Simultaneously in Terms of Time and Concentration

Attempts were then made to devise a method for expressing toxicity simultaneously in terms of both concentration and time. It was observed that increasing the concentration had about the same effect upon the kill rate as increasing the time. For example, in many instances the kill in 100 hours by a concentration of 25 ppm was the same as the kill in 25 hours by 100 ppm.

To calculate this time-concentration relationship, the logarithm of the square root of the product of time and concentration was plotted against percent kill. The point where the plot crossed the 50-percent kill line gave the value for the product of time and concentration required to kill half of the animals.

The time-concentration relationship was observed during a study of data obtained in assays of creosote and dinitrophenol and at the time was believed to be a general relationship. As other compounds were compared, however, it became apparent that the product of time and concentration is far from constant for many compounds and hope of arriving at a single number to represent the toxicity of a compound, for comparative purposes, was for the time abandoned.

Maximum Likelihood Solution of a Probit Plane

Methods were then sought to relate the variables time, concentration, and percent kill by an equation. The following equation of a probit plane, presented by Finney¹⁶ (1952), for relating percent kill to two dependent variables appeared to be a solution to the problem:

$$Y = a + bx + ct$$

where:

Y = probit = a function of the percent kill

x = log concentration

t = log time

a, b, and c are constants

The computational program developed from Finney's equation had the advantage that readings made at any set of times and concentrations could be employed to calculate the three constants of the equation. It was not necessary that the time and concentration schedules be the same for all compounds. Thus, even though changes had been made from time to time in these schedules, the computational program made it possible to determine the same three constants for all of the compounds tested.

Although a definition of toxicity, as given by Finney's equation, requires three constants, it is possible to employ a single number for special considerations. The most useful statistic which can be expressed as a single number is the concentration* required to kill half of the animals exposed continuously over a long period of time. These values can be employed to compare the toxicities of various compounds if the comparisons are restricted to the special purpose of screening potential wood preservatives. One hundred hours of continuous exposure were considered to constitute prolonged exposure and the three constants defining the toxicity of each agent were employed to calculate the LC-50's at 100 hours.

*The following symbols are used to denote lethal concentrations: "LC-50," lethal concentration - 50% kill. "MLC," minimum lethal concentration - 100% kill.

Data points where the kill is zero or one hundred percent are ordinarily difficult to handle. Finney outlined a procedure for handling such points. His procedure, which is a method of successive approximations, is called the Maximum Likelihood Method.

As the Maximum Likelihood Method is very time-consuming when hand calculators are employed, a program was prepared for processing the toxicity data on a digital computer. It was prepared under contract at the Data Processing Center, U. S. NAMTC, Point Mugu, California. Unless otherwise noted, the data of Appendix B was calculated by this program.

The data obtained in an assay of beta-naphthylamine are listed in Table 1. Reduction of this data by the digital computer program yielded the values -3.63, 2.55, and 3.22 for the constants a, b, and c. The value for the LC-50 at 100 hours as calculated from these constants was 7.13 ppm.

Multinomially Grouped Response Times

The assay that Finney employed as an example to illustrate the Maximum Likelihood Solution of a Probit Plane was one in which each reading was independent of all others. Only one reading had been made of each set of animals. In the Limnoria tests at this laboratory, however, each reading is not an independent observation, as several readings are made on each set of animals.

The inherent relationship of time, concentration, and percent kill should not be affected by the number of observations made on each group of animals, though the method for averaging or reducing the data might be slightly altered. If this fact were ignored, as it was in the program prepared for automatic computation, only slight errors would be made in estimating such a statistic as the LC-50. Estimates of the precision of the statistic, however, would be greatly altered.*

For example, a set of 16 readings corresponding to the percent kill at four times for each of four concentrations could be obtained with 16 dishes of animals, one reading being made for each dish. Or, a set of 16 readings could be obtained with four dishes of animals, four readings being made on each dish. The estimated equation relating time, concentration, and percent kill should be nearly the same in both cases; but the reliability of the results obtained in the experiment performed with only four dishes would be much less than that performed with sixteen. The program for automatic computation ignored this fact and assumed that each reading was an independent reading.

*For this reason and for other reasons to be mentioned in the discussion of reproducibility, these later estimates and fiducial limits have not been included in the table of experimental results in Appendix B.

Table I. Toxicity Input Data For Digital Computer
(Compound No. 821)

Time in Hours	Concentration in ppm	% Kill	% Kill among Controls
1.5	100	0	0.0
6.2	"	20	0.1
25	"	80	0.4
48	"	100	1.0
72	"	100	1.4
6.2	25	0	0.0
25	"	30	0.4
48	"	60	1.0
72	"	80	1.4
100	"	90	2.0
25	6.2	0	0.4
48	"	20	1.0
72	"	20	1.4
100	"	60	2.0
72	1.5	0	1.4
100	"	0	2.0

White and Graca¹⁷ (1958) have recently discussed the differences of these two types of observations. They described the readings in the first of the above examples as Binomially Grouped Responses and in the second as Multinomially Grouped Responses, and they described the appropriate mathematical treatment required to process the two types of data. For the first, a set of 16 independent readings of binomially spaced data define 16 simultaneous equations. For the second, a similar set of 16 readings of multinomially spaced data are not independent and are handled as four sets of four simultaneous equations with all of the readings at a single dose level processed as a unit.

A new program to process the toxicity data has just been completed at this laboratory. It embodies the mathematical procedure appropriate for Multinomially Grouped Responses. As in the former program, the Maximum Likelihood Method is employed, but this time the iterations are continued until no further change in the estimates¹⁸ (1954), is used.

Table II compares the values obtained by the two programs, the old program being based upon the binomial and the new upon the multinomial models. Values for the LC-50 are nearly identical, but the new program yields the appropriate estimates of error.

The new program can accommodate only 16 readings whereas the old program accommodated any number of readings. More than 16 readings were originally made for each of the last eight assays itemized in Table II. For these it was necessary to select four sets of readings. In the last eight assays, therefore, differences in the values obtained by the old and new methods do not arise solely because of differences in the mathematical treatments.

Data from the Teredo Larvae Tests

At first, the raw data in the Teredo tests consisted of a word description of the activity of the larvae at each of several time intervals for each of several concentrations. This raw data was employed to directly compare the toxicities of one compound to another. It was found, however, that a complete listing of the raw data was entirely too bulky and cumbersome to be useful in comparing or expressing toxicities of a large group of compounds.

Next, a semiquantal value chosen from a scale of ten was employed to describe the activity of the larvae in each beaker at each reading. Readings were made at 24, 48, and 72 hours. An attempt was made to reduce this data for each compound to a number somewhat resembling an LC-50 at 48 hours. This was defined

Table II. Comparison of the Old and New Computer Programs

Assay Number	Dilution Schedule	Number of Readings	LC-50 in 100 Hours as Calculated by	
			Old Program	New Program
984	Twofold	16	14.4	13.0 \pm 3.4*
2,158	Twofold	16	10.7	11.1 \pm 2.0
2,174	Twofold	16	6.3	6.2 \pm 1.8
2,189	Twofold	16	11.4	11.6 \pm 1.6
2,196	Twofold	16	21.7	23.4 \pm 2.5
2,204	Twofold	16	7.4	7.3 \pm 2.4
2,219	Twofold	16	9.1	9.3 \pm 1.9
821	Fourfold	24	7.1	6.6 \pm 2.7
843	Fourfold	24	19.4	21.5 \pm 6.0
897	Fourfold	20	9.9	13.8 \pm 4.4
952	Fourfold	24	8.8	8.4 \pm 2.9
966	Fourfold	24	15.9	14.6 \pm 5.1
1,025	Twofold	20	14.6	16.5 \pm 2.1
1,045	Twofold	20	18.4	14.9 \pm 2.2
2,097	Twofold	30	15.3	15.2 \pm 2.0

*Standard error of the LC-50 (lethal concentration - 50% kill)

as the concentration required to produce an effect in 48 hours comparable to a score of 5 on the semiquantal scale of ten. The so-called LC-50 at 48 hours was arrived at by interpolation. A careful review of the data indicated that these ratings were too subjective to be reliable.

The method finally chosen for reducing the data in the Teredo tests was to simply determine by inspection the minimum lethal concentration (MLC) that killed all of the larvae in 72 hours. Currently the observations are being continued considerably beyond the 72-hour readings and it is anticipated that in the future the 5-day reading will be employed as the basis for comparison.

Reproducibility

A given statistic obtained in any biological assay or chemical test should not be looked upon as "the value" for that statistic, but merely as "a value" obtained in a given attempt to measure it. Seldom, if ever, would exactly the same value be obtained if the experiment were repeated. In fact, the repeat values are frequently considerably different.

The results presented in Appendix B were, in the main, calculated from data gathered during the developmental period of the testing procedure. The values given for the LC-50's or the MLC's are therefore subject to more than the normal error and are not as reliable as the values which are being obtained at the present time.

In future reports it will be possible to make definite statements regarding the reproducibility of each result. In this report it is not practical to attempt to make such statements regarding each individual value. If the Limnoria test data were recalculated by the new program, a standard error for each LC-50 value could be obtained. However, the standard error, or rather the standard deviation of the LC-50, is not a measure of the reproducibility. It is merely a measure of the deviation of the various points from the equation and therefore the effort of reprocessing the data would not be worth the added information that would be obtained. A measure of the reproducibility of the results from week to week can only be made if each assay is repeated at least twice.

Some idea of the reproducibility of experimental results can be obtained by examining the data for creosote which was employed as a reference standard and was therefore tested many times. (See Table III). The fiducial limits were about 6 and 23 ppm. In about 95 percent of the assays the LC-50 should fall between these values.

Table III. Some Values Obtained in Assays of NCEL Standard Creosote
(LC-50's at 100 hours for 47 assays)

	<u>Log Values</u>	<u>Antilogs (ppm)</u>
Av. log LC-50	1.069	11.7
Av. log LC-50 +1 std. dev.	1.218	16.5
Av. log LC-50 -1 std. dev.	0.920	8.3
Av. log LC-50 +2 std. dev.	1.367	23.3
Av. log LC-50 -2 std. dev.	0.770	5.9

If the same variation is encountered in repeat assays of other agents, one would expect that 95 percent of the values for the LC-50 for a given compound would be from 1/2 to 2 times the average value for that compound. The range of plus or minus one standard deviation would be from about 0.7 to 1.4 times the average.

In spite of the uncertainty regarding the accuracy of any individual value given in this report, the over-all information is believed to be reliable. Attempts to overinterpret the data by classifying one compound as 10 percent or even 50 percent more toxic than another, would be wasted effort. With fair precision the data serves to distinguish the nontoxic compounds from the toxic and the extremely toxic from the moderately toxic. In its entirety, the data indicates which types of compounds are toxic and which types of chemical groupings render a compound toxic.

EXPERIMENTAL RESULTS AND THEIR SIGNIFICANCE

The detailed experimental data are assembled in Table VI, which appears in Appendix B. In the following discussion the significant experimental results are reviewed and analyzed. The sequence of this discussion parallels the presentation of toxicity data in Appendix B, with the headings keyed alphabetically to the Appendix Table VI.

A. Mixtures, Fractions and Derivatives of Coal-Tar Creosote

Several mixtures containing coal-tar creosote, fractions resulting from procedures such as chromatography and distillation, and samples of creosote that had been treated by a number of chemical reagents were obtained from various sources. Most of them were found to be toxic. Such a finding is compatible with the view that creosote is a mixture of many toxic ingredients rather than a solution of one or two toxic compounds in an inert solvent mixture.

Fractionation of creosote by chromatography is being investigated at this laboratory by Drisko and Hochman.¹⁹ They have recently succeeded in separating creosote into toxic and nontoxic fractions. Some of the chromatographic samples listed in Appendix B are from this study.

A series of distillation fractions of creosote were obtained from the Naval Research Laboratory through the courtesy of T. R. Sweeney and A. L. Alexander. Many of these were portions of the same samples employed in the experiments described by Price and Sweeney²⁰ (1956). Most of the fractions were toxic, some a little more toxic than creosote, some a little less. The two most volatile fractions and the essentially nonvolatile distillation residues were less toxic than the fractions of intermediate volatility. The solids precipitated by petroleum ether were also less toxic than the other portions of creosote.

These results suggest that creosote could be made more toxic by precipitating out some of the solids with petroleum ether and discarding those portions which distill up to 235 C at atmospheric pressure and the residues which boil above 210 C at a pressure of 15 millimeters.

Hydrogenation of creosote appeared to reduce and even abolish its ability to kill adult Limnoria and Teredo larvae. Treating creosote with mercuric acetate caused precipitation and the precipitated fraction probably contained most of the mercury. Just what merits, if any, mercurated creosote would have over creosote is difficult to assess from the toxicity data.

Because aliphatic constituents of creosote are considerably less toxic than the aromatic, toxicity tests should be a means of assessing the completeness of separation of the two components in a process of fractionation. β, β' oxydipropionitrile was employed by Heiks, Blum, and Burch²¹ (1954) to fractionate creosote into its aliphatic and aromatic constituents. The same procedure was employed at this laboratory and the toxicity data indicated that little or no separation occurred. The same conclusion was confirmed by spectral data.²²

Sea water can extract some of the toxic ingredients from creosote, but the distribution coefficients of the toxic ingredients are low enough to prevent a rapid loss of toxicity. Creosote extracted with sea water for several months suffered no observable loss in potency.

B. Compounds Found in Creosote and Coal-Tar

Toxicity tests have been run on about one-fourth of the nearly 200 compounds known to be constituents of creosote. More than half of the compounds tested were either nontoxic or only slightly toxic to marine borers. Most of the others were about as toxic as creosote itself and a few of them were several times more toxic. It is evident that the killing power of creosote cannot be ascribed to a single compound, but must be assigned to a combination of many toxic ingredients.

In view of the fact that only one-fourth of the compounds known to be present in creosote have been tested, the following statements regarding the relationship of the chemical structure and toxicity of the constituents of creosote are tentative.

1. Aliphatic Compounds

Most creosotes do not contain large quantities of aliphatic materials. Of the aliphatic compounds reported to be in creosote, only n-octadecane has been tested at this laboratory, and it was not toxic. A large number of aliphatic compounds not found in creosote were also tested. With the exception of derivatives of haloacetic acids, the only one which was toxic was formaldehyde and it was considerably less toxic than creosote. From this it was concluded that the aliphatic constituents of creosote do not contribute to its toxicity and, unless they have a desirable effect upon other properties, contribute nothing to the effectiveness of creosote as a wood preservative.

2. Monocyclic Compounds

Of the seventeen monocyclic constituents of creosote tested, only the dimethylphenols (xylenols) were sufficiently toxic to contribute significantly to the toxicity of creosote. The methylanilines (toluidines) and methylphenols (cresols) did

cause some kill at high concentrations, but the other monocyclic compounds were not toxic in concentrations up to 100 ppm. These included benzene, acetophenone, aniline, benzoic acid, phenol, mesitylene, picoline-N-oxide, pyridine, and pyridine-N-oxide. Because the dimethylphenols are fairly soluble in water and because the other monocyclic constituents of creosote were not very toxic, it is not likely that monocyclic compounds contribute much to the effectiveness of creosote as a preservative of marine piling.

3. Bicyclic Compounds

a. Phenyl-heterocyclic and phenyl-alicyclic compounds. Five compounds containing one phenyl group and one non-benzenoid ring structure were tested. Although none of them were toxic to Teredo larvae in the three-day test, they were all toxic to adult Limnoria with the exception of quinoline. Indole was the most toxic of the series, being several times as toxic to Limnoria as creosote. Although 8-methylquinoline and tetrahydronaphthalene were also toxic to Limnoria, they were only about a third as toxic as creosote.

Without information on the abundance of these compounds in creosote and without information of the toxicity of other phenyl-heterocyclic or phenyl-alicyclic constituents, it is difficult to assess their contribution to the over-all toxicity of creosote. But as indole is fairly soluble in sea water and other compounds of the series were only moderately toxic, it is not probable that this series of compounds contributes significantly to the effectiveness of creosote as a preservative of marine piling.

b. Derivatives of naphthalene. All of the bicyclic constituents of creosote and coal tar possessing a naphthalene parent ring structure (I - Figure 5) were toxic to both adult Limnoria and Teredo larvae. Acenaphthene, which contains a third ring in addition to the naphthalene ring, was not toxic. The naphthalenic compounds which possessed noncyclic ring substituents were from three to ten times as toxic as naphthalene itself, and from one to three times as toxic as creosote. The series included naphthalene, beta-naphthol, alpha-naphthylamine, alpha-methylnaphthalene, and beta-methylnaphthalene. The related tetrahydronaphthalene was less toxic than naphthalene.

This group of compounds certainly contributes significantly to the toxicity of creosote. However, many of the most toxic compounds of the series are appreciably soluble in water and the most abundant, naphthalene, was considerably less toxic than creosote. Also, naphthalene has an appreciable vapor pressure at room temperature and therefore slowly evaporates. Compounds in this series would be expected to contribute to the effectiveness of creosote in preserving marine piling during the early years but probably not to the lasting qualities of creosote.

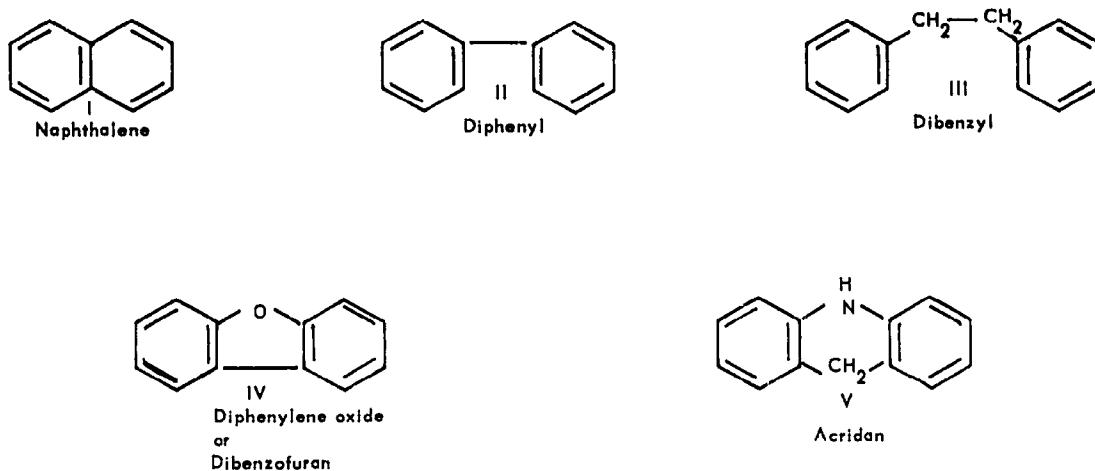


Figure 5. The structure of some toxic compounds found in creosote.

c. Diphenyl compounds. All of the bicyclic constituents of creosote tested which had the structure phenyl-x-phenyl were at least as toxic to marine borers as creosote and were, in general, more toxic. Diphenyl (II - Figure 5), dibenzyl (III - Figure 5), diphenylmethane, and the phenylphenols were among this group of compounds. Although the diazo compounds are not found in creosote, it is interesting to note that the only ones that were toxic were those having the structure phenyl-x-phenyl. Other compounds of this type are now under test and preliminary results indicate that most of them are also at least as toxic as creosote.

High toxicity in this series of compounds is of special interest because their vapor pressures at room temperature are a little lower than the vapor pressures of compounds in the naphthalene series. Also, most of these compounds are quite insoluble in sea water. They no doubt contribute both to the over-all toxicity of creosote and to its effectiveness as a wood preservative, at least during the early years.

4. Polycyclic Compounds

a. Diphenylene compounds. The diphenylene compounds were, in general, even more toxic than the diphenyl compounds which they resemble. Carbazole was an exception and was not toxic. Other diphenylene compounds which have been tested include fluorene, 2-aminofluorene, and dibenzofuran (IV - Figure 5). They were all highly toxic to both adult Limnoria and Teredo larvae. At present, several other compounds of this series are under test and preliminary results indicate that they too are highly toxic. Other workers have also found compounds of this type to be highly toxic to marine borers. For example, of over 80 compounds tested by White¹⁰ (1929), fluorenone was the most toxic to Limnoria lignorum.

Acridine, acridan (V - Figure 5), and a number of other compounds structurally related to acridine will soon be tested. Literature references indicate that they will also be highly toxic, as derivatives of acridine have been found to be toxic to a wide variety of animals and microorganisms, including Limnoria lignorum.

It would appear that compounds of this series contribute significantly to the over-all toxicity of creosote. As they are quite insoluble in sea water and have low vapor pressures at room temperature, they probably also contribute materially to the preservative effectiveness and lasting qualities of creosote.

b. Other polycyclic compounds containing three or more rings. With the exception of the diphenylene compounds, none of the polycyclic compounds containing more than two rings were toxic to Limnoria. The compounds of this series which were tested included acenaphthene, anthracene, phenanthrene, fluoranthene, pyrene, and triphenylmethane. Phenanthrene was very toxic to Teredo larvae, however, and tentative results of tests now under way indicate that possibly others of this series will be toxic to Teredo larvae when the test is extended to five days.

It is doubtful that compounds of this series contribute significantly to the over-all toxicity of creosote, at least not to Limnoria. Their low solubilities and vapor pressures would lead one to believe that these compounds would be long-lasting, but their low toxicity would not suggest that they contribute significantly to the effectiveness of creosote as a deterrent of Limnoria. Some of them, especially phenanthrene and fluoranthene, perhaps contribute to the long-lasting resistance of creosoted timbers to teredine borers.

C. Inorganic Compounds

Most of the inorganic compounds were tested before a reliable testing procedure had been developed and most of the results have therefore had not been reported in Appendix B. Testing of many of the inorganics was complicated because of solubility difficulties. Concentrated suspensions of insoluble organic compounds were usually more toxic than suspensions of low concentration. This did not hold true for the inorganics.

Tests of the toxicity of copper salts illustrate some of the complications which arose during the testing of the inorganics. When a few milliliters of a concentrated water solution of a salt such as copper chloride were added to sea water, a milky or opalescent colloidal suspension formed, which was probably copper carbonate. Concentrated suspensions were little or no more toxic than dilute suspensions. When sea water adjusted to a pH of 5.0 was employed for preparing the mixtures, clear solutions formed instead, and the more concentrated solutions were the more toxic. Copper salts were many times more toxic when the pH-five sea water was employed, although the pH-adjusted sea water was itself nontoxic. Mercury salts were considerably more toxic than salts of any other metal tested.

D. Organo-Metallic Compounds

1. Boron Compounds

Dodecyl boric acid, a fungistatic agent that is adsorbed to most surfaces, including wood, was not toxic to marine borers. Neither were nonyl boric acid or pyridine borane.

2. Copper Compounds

The organo-copper compounds were not very toxic to marine borers, probably because such compounds are so weakly dissociated. The concentration of copper ion is very low even though the concentration of organo-copper compound is high. This was well illustrated by the testing of mixtures of copper sulphate and 3(2'-hydroxy-5'-nitrophenylazo)-benzenesulfonic acid. The toxicity was about the same for mixtures of the copper salt and complexing agent in ratios of 1 to 2, 1 to 1, and 2 to 1. This feature should enhance the usefulness of organo-copper compounds by slowing down their rate of dissipation.

3. Mercury Compounds

The organo-mercury compounds were all very toxic, although a great deal of variation existed. Again, the degree of dissociation was perhaps responsible for the variation. The most toxic compounds were probably the most highly dissociated and therefore the least stable. Most of the organo-mercury compounds were also tested by Roe and Hochman²² (1959) in harbor tests at Port Hueneme and Pearl Harbor.

It was noted that many of the dyes had an affinity for wood and frequently even freely water-soluble dyes were encountered that clung tenaciously to wood fibers and could not be washed from them with sea water. It was reasoned that compounds combining the features of certain dyes and stains with the toxic qualities of mercury compounds might prove to be good wood preservatives, and the possibility was investigated.

The first of the mercury-containing dyes to be considered was merbromin. Merbromin was found to be very toxic but its affinity for wood was not as great as several of the basic dyes. Safranine, rosaniline, malachite green, and crystal violet were among the dyes which had the greatest affinity for wood. These and other dyes were mercurated and tested and all proved to be toxic to both Limnoria and Teredo larvae.

4. Tin Compounds

A number of organo-tin compounds were also tested. Although several of them were fairly toxic, they were considerably less toxic than the mercury compounds. Tributyltin compounds were the most toxic of this series of compounds.

E. Surface-Active Agents

Surface-active agents are known to have an affinity for cloth and lint particles and many of them can no doubt be adsorbed upon wood. As a number of them are known to be potent germicidal agents, the feasibility of employing surface-active agents as wood preservatives was investigated.

The toxicities of a large number of surface-active agents were determined, and most of them were found to be nontoxic to Limnoria. A few exceptions were found which were several times as toxic as creosote. The simple dimethyl alkyl amines may have been as much as ten times as toxic, but this conclusion was based upon assays of very crude or impure products. In general, the surface-active agents, especially the tertiary amines, were more toxic to Teredo larvae than to Limnoria. The quaternary agents were less toxic to Teredo larvae than were the tertiary amines.

Any quantity of a surface-active agent in excess of that bound to the wood through electrostatic forces is readily washed out of the wood. As the quantity of an agent which is adsorbed upon the fibers of wood is, in general, very small, only extremely toxic surface-active agents would be retained in sufficient quantities to be effective in protecting wood from marine borers. A few of the agents tested may have been sufficiently toxic to Teredo larvae to render timbers resistant to Teredine borer attack, but it is doubtful that any of them were sufficiently toxic to render wood resistant to Limnoria attack.

F. Pesticides

The chlorinated hydrocarbon insecticides were extremely toxic to Limnoria, endrin being more toxic than any other compound tested. Lindane, dieldrin, chlordane, and methoxychlor were nearly as toxic.

The extreme toxicities of these compounds were actually greater than the data in Table VI indicate. For uniformity, the LC-50 values reported in the table were calculated for 100 hours. Limnoria exposed to most concentrations of most poisons either die in less than 100 hours or they do not die at all. The LC-50 at 100 hours is usually nearly as small as at 200 hours. This was not true with endrin, dieldrin, DDT, lindane, chlordane, p-chlorophenyl-p-chlorobenzene sulfonate, or methoxychlor. A three- to five-day lag period generally occurred before Limnoria died from exposure to these agents. If the LC-50's had been calculated for 200 instead of 100 hours, the values would have been considerably smaller.

These same compounds were not very toxic to Teredo larvae, however. That is, they were not very toxic when the index of toxicity was the minimum concentration required to kill all of the test animals in 72 hours or less.

Other types of insecticides were also extremely toxic to Limnoria. Parathion of the organic phosphorus insecticides, pyrethrin of the naturally occurring insecticides, and N-methyl-1-naphthylcarbamate of the aromatics were nearly as toxic to Limnoria as the chlorinated hydrocarbons. They were moderately toxic to Teredo larvae.

Several pesticides were far less toxic to marine borers than had been expected. For example, pentachlorophenol, dichlorophene, and hexachlorophene were considerably less toxic to both Limnoria and Teredo than would have been predicted from their known toxicity to fungi. Benzylbenzoate, phenothiazine, fluoracetic acid, and a number of other pesticides were ineffective at concentrations as high as 100 ppm.

G. Extractives of Wood and Plants

None of the extracts of wood or plants were toxic to Limnoria. Several extracts and alkaloids have been isolated from greenheart by Hearst and Hochman²³ (1959). None of these were toxic to Limnoria, though they were moderately toxic to Teredo larvae. Tests of the greenheart alkaloids will be repeated in the near future, as testing procedures are now more reliable and the greenheart alkaloids are available in a purer state.

H. Haloacetic Acids and Their Derivatives

The toxicities of more than forty derivatives of haloacetic acids were determined. Most of these were furnished by Dr. Carl Wessel of the Prevention of Deterioration Center of the National Research Council, many of which were samples of the same preparations tested on fungi by Shirk and Gertler²⁴ (1958). Most of the samples were not crystalline and no tests of purity were made at this laboratory.

The toxicities of the haloacetates to Limnoria did not parallel their toxicities to fungi as reported by Shirk and Gertler. Their toxicities to Limnoria did, in general, parallel their toxicities to Teredo larvae, but it is difficult to make any exact statements regarding the Teredo larvae tests, as the results varied from week to week.

The fluoroacetates were for the most part less toxic to either organism than the chloroacetates were, and the chloroacetates were less toxic than the bromoacetates. Maximum potency was reached with the bromoacetates, with the iodoacetates a close second.

In their study of derivatives of bromoacetic acid, Shirk and Gertler found that the free acids were considerably less toxic to fungi than the esters were. This did not hold true in the tests on marine borers. Bromoacetic acid was, on the whole, slightly more toxic than its esters. Only one ester of iodoacetic acid was tested and it was less toxic to both species of test animals than the free acid or its salts. Chloroacetic acid was less toxic to Teredo larvae but was at least as toxic to Limnoria as its esters were. Fluoroacetic acid was essentially nontoxic to both Teredo larvae and adult Limnoria. Esters of fluoroacetic acid were not tested.

With the exception of four esters, the LC-50's of twenty derivatives of bromoacetic acid to Limnoria ranged from about 4 to 8 ppm. On the whole, the same derivatives were toxic to Teredo larvae in lower concentrations, but as has been stated, the results of the Teredo larvae test were quite erratic. The minimum lethal concentrations ranged from about 0.3 to 3.0 ppm.

With the exception of the n-2-butyloctyl ester, the LC-50's of the derivatives of chloroacetic acid averaged about 10 ppm, the range being from about 5 to 15 ppm. The minimum lethal concentrations required to kill Teredo larvae in 72 hours were also approximately 10 ppm for the derivatives of chloroacetic acid.

The n-2-butyloctyl esters of bromoacetic and chloroacetic acids were the least toxic of the derivatives of the corresponding acids to both Teredo larvae and Limnoria. In the work of Shirk and Gertler the same ester of bromoacetic acid was also one of the least toxic to fungi.

There was no evidence from the data on marine wood-boring organisms to refute the notion that all of the derivatives are toxic by virtue of their ability to liberate the free haloacids. The esters might hydrolyze either in the test dishes before entering the bodies of the test organisms, or enzymatically in the bodies of the organisms. Thus no evidence was presented to indicate that the esters of the haloacetic acids constituted a new class of poisons.

No lag period was associated with the onset of the toxic effects of the derivatives of the haloacetic acids. Usually, if the animals immersed in a solution of a given concentration of one of these compounds were not dead in a day or two, they would survive for a week.

Very little is known about the stability of the foregoing esters of haloacetic acids. Chloroacetic acid, however, is a fairly strong acid and one would expect that its esters would not be very stable in sea water, but would slowly hydrolyze. Amides of the corresponding acids would be expected to be considerably more stable, but as yet none have been tested.

I. Other Aliphatics

In addition to the surface-active agents, parathion, and the haloacetic acids, nearly forty noncyclic organic compounds of various types were tested. None of these other aliphatic compounds were sufficiently toxic to be worth mentioning. Even formaldehyde and formic acid, which are assumed to be general poisons, were essentially nontoxic to both Limnoria and Teredo larvae.

J. Organic Azo Compounds

Azobenzene is frequently employed as an acaracide, especially in greenhouses. It is also employed as a contact insecticide in the control of the corn borer. Azoxybenzene, a derivative of azobenzene, has been used successfully to control the larvae of the screwworm fly. These considerations warranted a study of the feasibility of employing azo compounds as wood preservatives.

Azobenzene was one of the first compounds assayed at this laboratory and was found to be very toxic to marine borers. Since then a large number of azo compounds and closely related oxidation and reduction products have been tested. Most of them were obtained from commercial sources, but a large number were synthesized at this laboratory. For the most part, these were known compounds and it was not necessary to prepare samples of analytical purity for elementary analysis. Thus, the toxicity tests were performed on samples of technical grade purity.

As most of the azo compounds were tested before many of the presently employed refinements had been incorporated into the toxicity testing procedure, the testing results are perhaps not as reliable as the results obtained in later assays. This shortcoming was not serious, however, as the variations in toxicity were quite large in this group of compounds. Several generalities relating structure to toxicity were readily apparent.

The presence of an azo group or related azoxy or hydrazo group does not in itself render a compound toxic; most of the compounds possessing these groups had no observable effect upon the animals. Thus, one cannot say that the azo compounds or the azoxy or hydrazo compounds in themselves represent a class of poisons. The compounds of the series which were poisonous must have been so because of some feature of the molecule other than the presence of the azo group.

In general, the most toxic members of the series were the simple unsubstituted compounds: azobenzene, hydrazobenzene, and azoxybenzene. Each of these consisted of two phenyl groups joined by an azo or closely related linkage.

A few compounds in which hydrogen atoms of the phenyl groups are replaced by a weakly ionized substituent were also very toxic. For example, p-methoxyazobenzene was as toxic as azobenzene itself. Phenylazophenol, phenylazoresorcinol, phenylazoaniline, and methyl-4-phenylazophenol (compounds in which phenyl hydrogens are replaced with weakly ionized phenolic or amino groups) were also quite toxic.

With the exception of one compound, all of the compounds in which the phenyl groups possess a highly ionized sulfonic acid radical were nontoxic. The exception, a new compound synthesized by Roe and Hochman²² and reported to have the structure 3(2-hydroxy-5-nitrophenylazo)-benzenesulfonic acid, was moderately toxic. Azo compounds possessing cyclic groups in excess of the two benzene rings were also nontoxic, and the compounds substituted with nitro groups were mostly nontoxic.

Some of the azo compounds have been tested by Hochman and Roe²² (1959) in harbor tests at Port Hueneme and Pearl Harbor.

K. Dyes and Stains

Testing of azo compounds led to a study of azo dyes, and several other events stimulated interest in dyes and stains in general. To begin with, a tri-phenylmethane dye, crystal violet, was reported by one of the earliest papers⁹ in the field to be toxic to marine borers, and many dyes were known to be toxic to microorganisms. Also, a fair proportion of them readily stained wood and were adsorbed to it quite firmly. Dyes and stains had the additional advantage of being water-soluble and could thus be applied to wood as aqueous solutions.

Malachite green was one of the first triphenylmethane dyes tested at this laboratory, and harbor tests conducted by Hochman and Roe²² (1959) proved it to be effective in retarding teredine borer attack for a long period of time, though it did not deter Limnoria.

The results of the tests of the toxicity of dyes and stains to marine borers were not encouraging as the dyes were in general not very toxic, especially to Limnoria. The dyes and stains available for testing were commercial products, and it must be remembered that for the past half century the dye industry has devoted itself not only to the task of finding dyes that have superior staining properties but in finding dyes that are safe for all types of human contact.

With the exception of crystal violet, none of the dyes or stains listed under K of Table VI were toxic to Limnoria and only a few of the azo dyes were toxic to either Limnoria or Teredo Larvae. These statements, of course, do not apply to the mercurated dyes listed under D of Table VI, which are toxic because of their mercury content. The triphenylmethane dyes and miscellaneous types are listed under K of Table VI, but most of the azo dyes have been included under J of Table VI.

Eight of the dyes and stains were toxic to Teredo larvae in very low concentrations and a number of others were toxic in moderately low concentrations. The relationship of chemical structure and toxicity of dyes and stains to Teredo larvae was compared. These comparisons were based upon limited data and therefore are of a tentative nature.

Many dyes are sulfonated to render them more freely soluble in water. With but one exception, none of these were toxic. For that matter, none of the dyes tested which were capable of forming sodium salts were toxic to Teredo larvae. On the other hand, with but two exceptions all of the basic dyes capable of forming salts with hydrochloric acid were toxic.

All but one of the compounds containing a dimethylamino group or diethylamino group were toxic. Most of these were basic dyes principally because of these groups. Of the eight dyes which were toxic to Teredo larvae in concentrations of 1-1/2 ppm or less, five contained the dimethylamino group.

No definite pattern relating type of dye to toxicity was noted. There were triphenylmethane dyes which were toxic and those which were not toxic, and the same statement applied for other types of dyes. No evidence was obtained to prove that the triphenylmethyl group in itself contributes to toxicity, and therefore the triphenylmethane dyes in themselves should not be looked upon as a class of poisons. There are some similarities in the type of dyes which are toxic to Teredo larvae and those which are toxic to Gram-positive bacteria.

L. Aromatic Nitro Compounds

Dinitrophenol has long been known as an agent which is very toxic to most forms of life. A number of other aromatic nitro compounds are also highly toxic and have found extensive use as disinfectants and pesticides.

Another feature for which aromatic nitro compounds are well known is that they can usually be readily identified. A number of highly reactive agents containing nitro groups have been developed for reacting with all types of organic compounds to form derivatives useful for identification purposes. The possibility of reacting these reagents with wood to incorporate a chemically bound preservative was considered. The success of such a preservative would depend upon the toxicity of aromatic nitro compounds to marine borers.

Many more nitro compounds were assayed than are listed under L of Table VI; some were more appropriately classified under different headings and appear in other sections of the table. The nitrophenols were all considerably more toxic than phenol itself. However, unless the aromatic nitro compounds possessed a phenolic or amino substituent, they were not toxic. It appears that nitro substituents on an aromatic ring do not in themselves render a compound toxic, but they do enhance the toxicity of phenols and amines. The toxicity of nitrophenols and amines to Limnoria parallel their toxicity to Teredo larvae.

M. Quinones and Hydroquinones

Most of the quinones and hydroquinones were of a low order of toxicity. Only quinone itself and two related compounds (2, 5-dimethyl-p-quinone and 2-methyl-1,4-naphthoquinone) were sufficiently toxic to be of interest from the standpoint of wood preservation. There was insufficient data from this class of compounds to permit statements to be made regarding the relationship of structure to toxicity.

N. Aromatic Amines and Phenols

As with compounds of several of the other classifications, there were a far greater number of aromatic amines and phenols tested than are listed under the respective class heading of Table VI. Many of them were listed under other groups of compounds, such as compounds found in creosote, pesticides, etc.

In almost all cases, the phenols and amines were more toxic than the parent ring structure devoid of the corresponding substituents. In general, phenols and amines in which the aromatic nuclei contained another substituent as well, were more toxic than the simple phenols and amines. In the anphthalene series, beta-naphthol and beta-naphthylamine were more toxic than the corresponding alpha compounds. The amino group of the highly toxic aminofluorene was also in the beta position.

O. Miscellaneous Organic Compounds and Mixtures

A number of miscellaneous organic compounds were tested which could not be classified under the groups which have been discussed. These were, in general, not very toxic and there is very little to say regarding the relationship of their structure to toxicity.

Indoxyl acetate was an interesting compound. Sea-water solutions of this toxic compound became blue as it was oxidized to an indigo dye. Indole, to which its structure is related, was also toxic. But other indigo dyes which were tested were not toxic.

GENERALIZATIONS CONCERNING TOXICITY TESTING

1. Toxicity tests are an effective device for screening potential protective materials, because these tests are relatively inexpensive and convenient and because such a small proportion of candidate materials actually prove to be sufficiently toxic to merit further investigation.

2. For the Limnoria tests, the most satisfactory index of toxicity that can be expressed as a single number is the concentration required to kill half of the animals (LC-50) after prolonged continuous exposure. Tests in which the index of toxicity is "time to kill at equal concentrations" rather than "concentration to kill in a given time" are not satisfactory. Tests in which the periods of exposure are of brief duration are also unsatisfactory.

3. When assays are repeated, the values for the LC-50, or other statistic, vary. In the Limnoria test, if the average LC-50 is taken as unity, the range of plus and minus one standard deviation will be from about 0.7 to 1.4 units. Ninety-five percent of the values will be from about 1/2 to 2 times the average.

TOXICITY TEST FINDINGS

1. It is possible to separate creosote into fractions, some of which are more toxic to marine borers than the original mixture. Hydrogenation of creosote reduces its toxicity.

With the exception of a few compounds (such as xylanol, indole, and phenanthrene), most of the toxic ingredients of creosote possess two benzene nuclei. Polycyclic compounds containing more than two benzene rings, compounds containing only one ring, and aliphatic compounds tend to be nontoxic or only slightly toxic to Limnoria. In general, the same statements apply to Teredo larvae, though a few of the larger polycyclic compounds are very toxic to this species.

2. Of the inorganic and organo-metallic compounds, those containing mercury are the most toxic to marine borers. Tributyltin complexes are also very toxic. Copper compounds are very toxic, but they are difficult to assay because they form insoluble precipitates with sea water.

3. Compounds possessing positively charged nitrogen atoms--such as greenheart alkaloids, basic organic dyes, and certain surface-active agents--are toxic to Teredo larvae in very low concentrations but not to adult Limnoria. With but one exception, all compounds possessing a negatively charged sulfonic acid group are nontoxic.

4. The chlorinated hydrocarbon insecticides are extremely toxic to Limnoria, endrin and lindane being more toxic than any other compounds known. Deaths do not occur until one or two hundred hours after exposure to these agents, but extremely small quantities are lethal. The same compounds are not very toxic to Teredo larvae, however. Other extremely toxic insecticides are parathion, pyrethrin, and N-methyl-1-naphthyl carbamate.

5. Fungicidal activity does not parallel toxicity to marine borers as closely as does insecticidal activity. For example, the highly effective fungicidal agents pentachlorophenol, dichlorophene, and hexachlorophene are only moderately toxic to marine borers.
6. With the exception of fluoroacetic acid, haloacetic acids and their esters are very toxic to marine borers. Their toxic effects are exerted very rapidly and deaths occur in a short time. The fluoroacetates are less toxic than the chloroacetates, and the chloroacetates less toxic than the bromoacetates. Maximum potency is reached with the bromoacetates, the iodoacetates being slightly less toxic. The toxicity of the haloacetates to adult Limnoria parallels their toxicity to Teredo larvae.
7. A number of aromatic azo compounds are very toxic, but the presence of an azo group does not in itself render a compound toxic to marine borers. Some of the more toxic azo or related compounds are azobenzene, azoxybenzene, hydrazobenzene, phenylazoaniline, methoxyazobenzene, and methoxyazoxybenzene. The most toxic compounds of the series possess two and only two benzene nuclei that are relatively free of substituent groups.
8. Nitro groups enhance the toxicity of phenols, but the presence of a nitro group does not in itself render an aromatic compound toxic.
9. As a class of compounds, quinones and hydroquinones are not very toxic to marine borers, though toxic quinones are found.
10. Aromatic amines and phenols vary considerably in toxicity. Some of the most toxic of the aromatic amines and phenols are compounds containing two benzene nuclei, such as 2-amino-fluorene, beta-naphthylamine, beta-naphthol, and phenyl-phenol. Nitrophenols and dimethyl phenols are also very toxic to marine borers, though they possess only one benzene nucleus.

CONCLUSIONS

Many chemical compounds are far more toxic than creosote to marine borers. Some compounds are approximately as toxic to adult Limnoria as to Teredo larvae. A number of compounds, however, are much more toxic to one species than to the other, some being more toxic to adult Limnoria and others more toxic to Teredo larvae.

RECOMMENDATIONS

1. Toxicity testing should be continued.
2. The Table of Appendix B should be used as a guide in the selection of compounds for further testing. Those compounds which are not toxic to marine borers should be eliminated from further consideration and those which are toxic should be investigated by additional testing procedures. Other factors such as solubility in sea water, resistance to chemical change, affinity for wood fibers, and volatility should also be considered before a potential wood preservative is tested in the harbor.
3. Some materials which are particularly recommended for harbor testing are:
 - a. As teredine borer deterrents:
Basic organic dyes such as malachite green, safranine, gentian violet, and rosaniline
Alkaloids from greenheart
Phenanthrene
 - b. As Limnoria deterrents:
Chlorinated hydrocarbon insecticides such as endrin and lindane
 - c. As general marine borer deterrents:
Creosote
Constituents of creosote containing 2 benzene nuclei such as biphenyl, fluorene, and dibenzofuran
Mixtures of two or more agents such as creosote, phenanthrene, basic organic dyes, endrin, lindane, biphenyl, fluorene, and dibenzofuran
Mercurated dyes
Organometallic compounds
4. Studies on the fractionation of creosote should be continued. Attempts should be made to separate creosote into aliphatic, saturated, monocyclic, bicyclic, and polycyclic fractions.

FUTURE PLANS

Emphasis will be placed upon determining the relationship between chemical structure and the toxicity of compounds found in creosote. The mode of action and possible enzyme inhibition mechanisms will be studied. Further investigation

of newer pesticides, organo-tin compounds and compounds possessing positively charged nitrogen atoms will also be emphasized. A study will be made of the various factors influencing the results in the Teredo larvae test and attempts will be made to design a more satisfactory procedure for evaluating toxicity towards this organism.

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APPENDIX A

STANDARD PROCEDURE FOR EVALUATING THE TOXICITY OF CHEMICALS TO *LIMNORIA TRIPUNCTATA*

Test Animals

The Limnoria tripunctata are collected at least one week in advance of the start of the assay and stored in dishes of clean sea water containing several splinters of wood which have been previously well soaked in sea water. The test animals are examined periodically during the several days preceding their use and dead animals, injured animals, or animals not actively boring on the splinters are discarded.

Preliminary Tests

A screening process precedes the actual assay. Solutions or suspensions of the test compounds are prepared in sea water at a concentration of 100 ppm. Ten milliliters of each solution are added to small petri dishes each containing ten Limnoria, only one dish of animals being employed for each compound tested. Compounds which kill half or more of the animals in 200 hours or less are selected for further testing by the procedures outlined below.

Preparation of the Test Dishes

Sixty dishes are employed for each series of toxicity tests that are performed. Petri dishes of standard 60-millimeter diameter are used. These are partitioned into two compartments by two small cylinders or rings cut from 20-millimeter glass tubing. A few days before the start of the assay, ten animals are added to each dish, with five animals in each compartment (Figure 6). A small splinter of wood, presoaked in sea water, is also placed in each compartment. Movement of the Limnoria is restricted to an area that can be conveniently viewed under a microscope, but the test solutions flow freely to all parts of the test dishes.

On the morning that the assay is to begin, each dish is examined under a magnification of about 20 times, and dead or injured animals are replaced. The sea water is removed by means of a transfer pipette fitted with a small rubber bulb, and the test solutions are introduced. The test solutions must be added before the dishes dry out, but the animals will survive out of water for at least a half hour if the dishes are covered.



Figure 5. The film employed to evaluate the toxicity of the plant (see Fig. 4).

Concentration Schedule

Each preservative mixture or other chemical agent assayed is tested at four concentrations spaced at proportionately equal distances. Usually each succeeding concentration is twice that of the next lower in the series. However, it is sometimes desirable to cover a broader range of concentrations than can be covered by the twofold schedule, and a fourfold or even greater dilution schedule may be employed. An accompanying decrease in accuracy occurs with the higher dilution schedules and once the approximate range of the concentration required to kill half of the animals (LC-50) in 200 hours is known, the assay is repeated at the twofold dilution schedule in concentrations bracketing the LC-50.

Preparation of Solutions for Assay

Stock solutions of each chemical to be tested are prepared in concentrations 200 times greater than the desired concentration of the test chemicals in sea water. The solvents selected for preparing these stock solutions are redistilled acetone, ethyl alcohol, methyl cellosolve, or water. To prepare the test solutions or suspensions, 1 milliliter of each of the appropriate stock solutions are added to 200 milliliters of sea water, and the resulting mixtures are used before they have had time to settle. Sea-water controls are prepared by adding 1 milliliter of the solvent of choice to 200 milliliters of sea water.

Thus, the test solutions not only contain the test compounds but also contain an organic solvent at a concentration of 1/2 percent. Half-percent solutions of the recommended solvents in sea water have no observable effect upon the well-being of Limnoria in 200 hours.

The pH of the sea water is seldom altered sufficiently to be detected. The pH is always checked, however, and in the few instances where it is found to be greater than 7.8 or less than 6.8, adjustments are made.

Compounds Insoluble in the Permissible Solvents

When the compounds which are insoluble in acetone, ethyl alcohol, methyl cellosolve, or water are tested, each must be dealt with as an individual problem. Fortunately, not too many of these compounds are encountered. Chelates and synthetic resins are the principal examples.

One test that can be used is to merely determine the effect of saturated solutions. A pinch or a drop of the material can be added directly to one or two dishes of Limnoria in sea water. If the solutions are not toxic, further testing of

the compound is unnecessary. In these instances, the only statement that can be made regarding their toxicity is that saturated solutions are not toxic.

Several solvents that are immiscible with sea water are only slightly toxic to Limnoria. These solvents can be employed in conjunction with nontoxic surface-active agents if adequate consideration is given controls; or aliquots of the immiscible solvent mixtures can be pipetted directly into dry petri dishes. After the solvent has evaporated, sea water and animals can be added to the dishes.

In many cases, solution in water may be affected by the use of sodium hydroxide or hydrochloric acid and the pH of the sea water mixtures must be adjusted to neutrality.

Reference Standard

A reference standard is used in every set of assays performed. Coal-tar creosote (Grade One, A.W.P.A. specification) was chosen as the primary standard because it is the standard preservative of marine piling. Creosote is usually tested at concentrations of 50, 25, 12-1/2, and 6-1/4 ppm.

Scrambling of the Petri Dishes

To insure that the observer's personal opinion does not influence the scores assigned, a scrambling system is used by which (1) the dishes of test animals are assigned to the solutions in random order, and (2) observations of the percent kill are made in random order with respect to compound and concentration.

The dishes containing the Limnoria are numbered from 1 through 60 with arabic numerals in the order of their position on the workbench. A scramble form (Table IV) is prepared by ruling off four columns and fifteen rows of squares. The columns are labeled A, B, C, and D to correspond to the four concentrations and the fifteen rows are labeled I through XV to correspond to the thirteen test compounds, the creosote standard, and the sea-water control. Arabic numbers from 1 through 60 are then assigned to the sixty squares in a random order by means of a well-shuffled deck of 60 numbered cards. Finally, 10 milliliters of each of the test solutions are added to the petri dish indicated on the scramble form.

Observations of the Percent Kill

In all experiments the same time intervals are chosen for counting the dead animals. The times selected for these observations are 25, 50, 100, and 200 hours from the time the animals were first covered with the test solutions. Independent counts of the number of animals dead in each dish are made by two observers and the results are averaged.

Table IV. Sample Scramble Form

Chemical Agent	NCEL Number	Compound Number	A	B	C	D
Creosote, fraction 70-376-94	2191	I	8	25	52	55
N.R.L. special creosote, frac. 2	2192	II	60	40	57	36
N.R.L. creosote, hydrogenated	2193	III	24	1	31	41
Creosote, residue of hydrogenated	2194	IV	29	7	13	4
Benzylamine	2195	V	50	15	51	35
Dibenzylamine	2196	VI	2	58	28	48
Tribenzylamine	2197	VII	44	33	21	38
Diphenylamine	2198	VIII	56	23	6	9
English coke oven tar creosote	2199	IX	43	17	12	19
Triphenylamine	2200	X	22	37	16	46
Benzoin	2201	XI	27	47	32	39
Wetting agent	2202	XII	5	3	11	49
Creosote plus wetting agent	2203	XIII	10	34	59	53
Creosote standard	2204	XIV	30	18	45	54
Controls	2205	XV	26	20	14	42

Limnoria are unable to live in sea water which is stagnant and full of decaying dead animals. Dead and decomposing Limnoria are, therefore, occasionally removed from the test dishes. When this is done a notation is made on the scoring sheet so that the observer may add these animals to his count when the next reading is made.

Duplicate Assays

Each compound whose toxicity is about as great as or greater than that of creosote is assayed twice, the two assays being performed on different weeks. Tests of the agreement of results on different weeks is thus made possible. Also, when the assays are repeated on different weeks it becomes possible to compare the variance of the LC-50's to the variance of the creosote coefficients. As present it is not known which of these two statistics is most reproducible. Assays of compounds which are not toxic or which are only slightly toxic are repeated, but only at a single concentration of 100 ppm.

Report of Assay

Table V illustrates the manner in which the data is finally recorded.

Table V. Results of Assay

Name of Chemical:		NCEL Number: 2196									
Dibenzylamine											
LC-50 at 200 hours		Fiducial limits (95%)		LC-50 Creosote: 2.97							
Estimated: 17 ppm Calculated: 15.8 ppm		Upper: 19.2 ppm Lower: 12.4 ppm		Creosote coefficient: 0.188							
Where $Y = \text{logit of \% kill} = a + b \log_e \text{conc.} + c \log_e \text{time}$ $a = -19.1, b = 3.32, c = 1.88, n = b/c = 1.77$											
Comments: Acetone was employed as the dispersing solvent.											
PERCENT KILL											
Code	Concen- tration	Date				pH	Nature of Mixture or Solution				
		12-16	12-17	12-19	12-23						
		25 hours	50 hours	100 hours	200 hours						
2	A 50 ppm	50%	55%	100%	100%	7.62	clear				
58	B 25 ppm	10%	20%	60%	100%						
28	C 12.5 ppm	10%	10%	20%	30%						
48	D 6.2 ppm	0%	0%	0%	0%						
	Control	0%	0%	0%	0%	7.35	clear				

APPENDIX B

TABLE OF TOXICITIES

Classification

The compounds in Table VI were divided into fifteen classes designated by the letters A through O. Many compounds could be placed appropriately into several of these classifications. In such instances, the compounds were usually listed with the first class into which they could appropriately be classified. For example, phenol could be listed under Class B, "Compounds Found in Creosote and Coal Tar." Or it could be listed under Class N, "Aromatic Amines and Phenols." It was listed only under Class B, the first appropriate class to appear in the table.

NCEL Numbers

Strictly speaking, the NCEL numbers listed in Table VI simply designate a notebook location. Nevertheless, the NCEL numbers help to describe the procedures employed in the respective *Limnoria* tests. Tests designated by numbers greater than 700 are chronological. Records of the first tests performed were kept in notebooks I and II and the NCEL numbers used to indicate these tests are preceded by a Roman numeral I or II.

Tests indicated by an NCEL number preceded by a Roman numeral I were the very earliest performed. In these tests, observations of the percent kill (in the *Limnoria* test) were made at 24, 48, and 72 hours. To obtain values for the kill at 100 hours, it was necessary to extrapolate. Mixed populations of *Limnoria tripunctata* and *Limnoria quadripunctata* were used as test animals and the death rate among the controls was significant. The results of these tests are therefore less reliable than the results of tests performed at a later date.

Tests indicated by an NCEL number preceded by a Roman numeral II were performed at a subsequent date. A single species, *Limnoria tripunctata*, were employed as test animals. As readings were extended to 100 hours in these tests, extrapolations were not necessary. However, deaths among control animals were still quite high and creosote standards were not run for comparison.

In tests indicated by NCEL numbers from 700 to 1000, observations of the percent kill were made at 1-1/2, 6-1/4, 25, 50, 72, and 100 hours. In most of these tests a creosote standard was tested simultaneously. Because improved techniques were employed in selecting test animals, the death rate of control animals was negligible.

Starting with test number 1000, the scramble system was instituted and duplicate observations were made. Two observers made independent readings at each time interval and the two scores were averaged. Thus, the greatest reliance can be placed upon the tests indicated by NCEL numbers greater than 1000.

Specific Examples Illustrating the Use of Table VI

A-4, creosote distillation fraction 1, has an NCEL number greater than 1000. This indicates that most of the improvements in testing technique were incorporated in the procedure for testing this agent. The data was calculated by the digital computer program and the value 47.2 ppm was obtained for the LC-50 at 100 hours. A value of 16.9 ppm was obtained for the LC-50 of a sample of standard creosote tested simultaneously. To Limnoria, the agent was therefore about one-third as toxic as NCEL standard creosote. In the Teredo larvae test for A-4, the minimum concentration in which all of the Teredo larvae were dead at the end of 72 hours was greater than 100 ppm. That is, 100 ppm was the highest concentration tested and in none of the dishes were all of the Teredo larvae dead at the end of 72 hours. For creosote run simultaneously, the minimum concentration that killed all of the Teredo larvae in 72 hours was 25 ppm.

Skipping to J-28, the NCEL numbers are preceded by a Roman numeral I, indicating that the tests were among the earliest performed. As the observations at that time were made at 24, 48, and 72 hours only, it was necessary to extrapolate to arrive at a value for the kill at 100 hours. For convenience, the report of two tests of the same compound is included in the same line. In the first test, the results indicated that the LC-50 was about 100 ppm. The asterisk indicates that the value was arrived at merely by inspection of the data rather than by the digital computer program. In the second test, the LC-50 was greater than 100; that is, less than half of the animals were killed even by the highest concentration tested. A creosote standard was not included in the tests performed that week and no Teredo larvae test was performed.

In the Teredo larvae test for F-16, all the larvae in all the dishes were dead at the end of 72 hours.

Table VI. Toxicity of Chemical Agents to Marine Borers

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-1 - Creosote, NCEL standard (Average for many tests)	11.7	--	25	--
A-2 - NCEL No. 1059 Creosote, chromatographic residues consisting mainly of tars and phenols	6.2	21.5	< 6.2	25
A-3 - NCEL No. 1058 Creosote, chromatographic fraction consisting mainly of aromatic hydro- carbons	10.5	21.5	25	25
A-4 - NCEL No. 1122 Creosote, distillation fraction 1, material boiling below 200° @ 760 mm	47.2	16.9	> 100	25
A-5 - NCEL No. 1101 Creosote, distillation fraction 2, boiling range: 200° - 235° @ 760 mm	29.5	16.9	> 100	25
A-6 - NCEL No. 1102 Creosote, distillation fraction 3, boiling range: 235° @ 760 mm to 130° @ 15 mm	9.2	16.9	100	25
A-7 - NCEL No. 1103 Creosote, distillation fraction 4, boiling range: 130° - 160° @ 15 mm	3.2	16.9	25	25
A-8 - NCEL No. 1104 Creosote, distillation fraction 5, boiling range: 160° - 190° @ 15 mm	10.6	16.9	100	25

Table VI. (Cont'd)

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-9 - NCEL No. 1105 Creosote, distillation fraction 6, boiling range: 190 - 210° @ 15 mm	10.3	16.9	100	25
A-10 - NCEL No. 1106 Creosote, distillation residue, boils above 210° @ 15 mm	> 100	16.9	100	25
A-11 - NCEL No. 1107 Creosote, depleted of distillation fraction 2	13.9	16.9	100	25
A-12 - NCEL No. 1108 Creosote, depleted of distillation fraction 3	28.0	16.9	25	25
A-13 - NCEL No. 1109 Creosote, depleted of distillation fraction 4	29.3	16.9	25	25
A-14 - NCEL No. 1110 Creosote, depleted of distillation fraction 5	17.8	16.9	25	25
A-15 - NCEL No. 1111 Creosote, fractions boiling below 210° @ 15 mm	18.9	16.9	25	25
A-16 - NCEL No. 1112 Creosote, distillation fraction 2 (65%), distillation residue (35%)	47.8	16.9	25	25

Table VI. (Cont'd)

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-17 - NCEL No. 1113 Creosote, distillation fractions 3 and 4 (65%), distillation residue (35%)	11.0	16.9	25	25
A-18 - NCEL No. 1114 Creosote, distillation fraction 5 (65%), distillation residue (35%)	18.5	16.9	> 100	25
A-19 - NCEL No. 1115 Creosote, distillation fraction 6 (65%), distillation residues (35%)	17.9	16.9	> 100	25
A-20 - NCEL No. 1116 Creosote, petroleum ether soluble fraction	19.8	16.9	25	25
A-21 - NCEL No. 1117 Creosote, petroleum ether soluble fraction, tar acids removed	19.0	16.9	25	25
A-22 - NCEL No. 1118 Creosote, petroleum ether soluble fraction, tar bases removed	19.3	16.9	25	25
A-23 - NCEL No. 1119 Creosote, petroleum ether soluble fraction, tar acids and tar bases removed	16.3	16.9	25	25
A-24 - NCEL No. 1120 Creosote, reconstituted	16.7	16.9	25	25

Table VI. (Cont'd)

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-25 - NCEL No. 1121 Creosote, solids precipitated from creosote by petroleum ether	44.4	16.9	25	25
A-26 - NCEL No. 1124 Creosote, NRL standard	16.8	16.9	25	25
A-27 - NCEL No. 1125 Creosote (70%); coal tar (30%)	17.4	16.9	25	25
A-28 - NCEL No. 966 & 1017 Creosote, hydrogenation of. A. Before treatment with hydrogen. Sp gr = 1.088	24.1	24.1	25	25
A-29 - NCEL No. 963 Creosote, hydrogenation of. B. Treated once with hydrogen. Sp gr = 1.038	19.0	24.1	25	25
A-30 NCEL No. 965 Creosote, hydrogenation of. C. Treated once with hydrogen. Sp gr = 1.055	22.6	24.1	25	25
A-31 - NCEL No. 964 Creosote, hydrogenation of. D. Treated twice with hydrogen. Sp gr = 1.035	27.0	24.1	25	25
A-32 - NCEL No. 996 Creosote, hydrogenation of. E. Treated three times. Sp gr = 0.992	100	24.1	25	25

Table VI. (Cont'd)

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-33 - NCEL No. 856 Creosote, mercurated with mercuric acetate, precipitated fraction	8.2	9.3	25	25
A-34 - NCEL No. 855 Creosote, mercurated with mercuric acetate, supernatant fraction	15.0	9.3	25	25
A-35 - NCEL No. 1013 Creosote, oxydipropionitrile soluble fraction (first trial)	27.1	32.3	< 12.5	25
A-36 - NCEL No. 1043 Creosote, oxydipropionitrile soluble fraction (second trial)	22.0	18.5	< 6.2	12.5
A-37 - NCEL No. 1014 Creosote, oxydipropionitrile insoluble fraction (first trial)	26.0	32.3	< 12.5	25
A-38 - NCEL No. 1042 Creosote, oxydipropionitrile insoluble fraction (second trial)	14.4	18.5	< 6.2	12.5
A-39 - NCEL No. 2015 Creosote, neutral residue after removal of tar acids and bases	11.1	14.7	< 6.2	12.5
A-40 - NCEL No. 1022 Creosote, polynuclear hydrocarbons and tar bases regenerated from creosote picrate	13.7	14.6	< 12.5	< 6.2

Table VI. (Cont'd)

A. Mixtures, Fractions, and Derivatives of Coal-Tar Creosote

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
A-41 - NCEL No. 997 Creosote, sea water extracted	8.7	5.7	6.2	<12.5
A-42 - NCEL No. 2016 Creosote, tar acids from	17.3	14.7	< 6.2	12.5
A-43 - NCEL No. 2017 Creosote, tar bases from	15.5	14.7	< 6.2	12.5
A-44 - NCEL No. 1015 Coal tar	68.9	32.3	25	25
A-45 - NCEL No. 842 Lindane-creosote mixture (50:50)	0.80	19.4	6.2	25

Table VI. (Cont'd)

B. Compounds Found in Creosote and Coal Tar

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
B-1 - NCEL No. 804-2119 Acenaphthene	98.1	9.0	25	25
B-2 - NCEL No. 789 Acetophenone	> 100	12.6	--	--
B-3 - NCEL No. 793 Ammonium thiocyanate	> 100	14.6	> 100	25
B-4 - NCEL No. 597-827 Aniline	> 100	11.0	> 100	25
B-5 - NCEL No. 799 Anthracene	> 100	18.2	> 100	25
B-6 - NCEL No. 2110 Benzene	> 100	9.0	> 100	12.5
B-7 - NCEL No. 817 Benzoic acid	> 100	--	100	--
B-8 - NCEL No. 2123 Bibenzyl	1.1	9.0	12.5	12.5
B-9 - NCEL No. 2120 Biphenyl	8.1	9.0	12.5	12.5
B-10 - NCEL No. 794 Carbazole	> 100	14.6	> 100	25

Table VI. (Cont'd)

B. Compounds Found in Creosote and Coal Tar

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
B-11 - NCEL No. I-594 <u>o</u> -Cresol	100*	--	> 100	--
B-12 - NCEL No. I-596 <u>m</u> -Cresol	100*	--	100	--
B-13 - NCEL No. I-595 <u>p</u> -Cresol	100*	--	25	--
B-14 - NCEL No. 2132 Dibenzofuran	< 6.2	9.0	12.5	12.5
B-15 - NCEL No. I-601 2,6-Dimethylphenol	9.5	--	> 100	--
B-16 - NCEL No. I-600 3,5-Dimethylphenol	7.2	--	100	--
B-17 - NCEL No. 2121 Diphenylmethane	17.8	9.0	12.5	12.5
B-18 - NCEL No. 2131 Fluoranthene	> 100	9.0	--	--
B-19 - NCEL No. 2116 Fluorene	11.6	9.0	12.5	12.5
B-20 - NCEL No. 736 Indole	5.3	--	> 100	--

*Estimated

Table VI. (Cont'd)

B. Compounds Found in Creosote and Coal Tar

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
B-21 - NCEL No. 816 Isoquinoline	34.2	--	100	25
B-22 - NCEL No. 2125 Mesitylene	>100	9.0	>50	12.5
B-23 - NCEL No. 2133 1-Methylnaphthalene	8.9	9.0	50	12.5
B-24 - NCEL No. 2130 2-Methylnaphthalene	24.3	9.0	--	--
B-25 - NCEL No. 813 8-Methylquinoline	61.6	8.7	100	25
B-26 - NCEL No. 731 Naphthalene	50*	--	>100	--
B-27 - NCEL No. 2104 Naphthalene	41.7	11.6	100	<12.5
B-28 - NCEL No. 2118 Naphthalene	20.8	9.0	--	--
B-29 - NCEL No. 819 α -Naphthol	12.9	--	6.2	25
B-30 - NCEL No. 820 β -Naphthol	10.5	11.0	6.2	25

*Estimated

Table VI. (Cont'd)

B. Compounds Found in Creosote and Coal Tar

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
B-31 - NCEL No. 767 <i>n</i> -Naphthylamine	20.6	--	< 1.5	25
B-32 - NCEL No. 821 <i>β</i> -Naphthylamine	7.1	11.0	25	25
B-33 - NCEL No. 802 <i>n</i> -Octadecane	> 100	18.2	> 100	25
B-34 - NCEL No. 800 Phenanthrene	67.2	18.2	< 1.5	25
B-35 - NCEL No. 2115 Phenanthrene	80*	--	--	--
B-36 - NCEL No. I-593 Phenol	> 100	--	> 100	--
B-37 - NCEL No. 818 <i>o</i> -Phenylphenol	9.1	--	25	25
B-38 - NCEL No. I-588 4-Picoline-N-oxide	> 100	--	--	--
B-39 - NCEL No. 2117 Pyrene	> 100	9.0	> 100	< 12.5
B-40 - NCEL No. I-587 Pyridine-N-oxide	100	--	--	--

*Estimated

Table VI. (Cont'd)

B. Compounds Found in Creosote and Coal Tar

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
B-41 - NCEL No. 790 Quinoline	> 100	12.6	--	--
B-42 - NCEL No. 814 1,2,3,4-Tetrahydronaphthalene	62.4	8.7	> 100	25
B-43 - NCEL No. 2124 1,2,3,4-Tetrahydronaphthalene	100*	9.0	50	12.5
B-44 - NCEL No. 2109 Toluene	> 100	9.0	> 100	12.5
B-45 - NCEL No. 795 <u>o</u> -Toluidine	> 100	14.7	100	25
B-46 - NCEL No. 801 <u>m</u> -Toluidine	29.5	18.2	> 100	25
B-47 - NCEL No. 791 <u>p</u> -Toluidine	25*	12.6	--	--
B-48 - NCEL No. 2122 Triphenylmethane	> 100	9.0	12.5	< 12.5
B-49 - NCEL No. 2111 <u>o</u> -Xylene	> 100	9.0	100	12.5
B-50 - NCEL No. 2112 <u>m</u> -Xylene	> 100	9.0	100	12.5
B-51 - NCEL No. 2113 <u>p</u> -Xylene	> 100	9.0	100	12.5

*Estimated

Table VI. (Cont'd)

C. Inorganic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
C-1 - NCEL No. I-589 Ammonium bifluoride	> 100	--	--	--
C-2 - NCEL No. II-687 Arsenious acid	10*	--	25	--
C-3 - NCEL No. 718 Cesium chloride	> 100	--	--	--
C-4 - NCEL No. 710 Chromium potassium sulfate	> 100	--	> 100	--
C-5 - NCEL No. 720 Germanium dioxide	> 100	--	--	--
C-6 - NCEL No. 772 Hydroxylamine hydrochloride	50*	--	>100	--
C-7 - NCEL No. 725 Indium trichloride	> 100	--	--	--
C-8 - NCEL No. 719 Lanthanum chloride	> 100	--	--	--
C-9 - NCEL No. I-53 Manganous nitrate	> 100	--	--	--
C-10 - Mercuric sulfide	> 100	--	>100	--

*Estimated

Table VI. (Cont'd)

C. Inorganic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
C-11 - NCEL No. 1039 Molybdic acid, sodium salt	> 100	18.4	> 100	--
C-12 - NCEL No. 726 Neodymium chloride	> 100	--	--	--
C-13 - NCEL No. 715 Palladium (ous) chloride	> 100	--	> 100	--
C-14 - NCEL No. 716 Rubidium chloride	> 100	--	> 100	--
C-15 - NCEL No. 845 Sodium azide	77.7	11.7	> 100	25
C-16 - NCEL No. 869 Sodium silicofluoride	> 100	7.8	> 100	25
C-17 - NCEL No. 717 Thallium (ous) chloride	50*	--	100	--
C-18 - NCEL No. 724 Yttrium nitrate	> 100	--	--	--
C-19 - NCEL No. 831 Zinc sulfate	> 100	12.3	100	25

*Estimated

Table VI. (Cont'd)

D. Organo-Metallic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
D-1 - NCEL No. 2046 Dodecylboric acid	> 50	14.2	< 6.2	--
D-2 - NCEL No. 2047 Nonylboric acid	> 50	14.2	12.5	--
D-3 - NCEL No. 2066 Pyridine borane	> 100	20.6	> 100	--
D-4 - NCEL No. 2013 Copper chelate of kojic acid	> 100	14.7	< 6.2	--
D-5 - NCEL No. 1000 Copper pentachlorophenate	> 100	--	> 100	--
D-6 - NCEL No. 763 Copper 3-phenylsalicylate	> 100	--	100	--
D-7 - NCEL No. 753 Copper phthalocyanine (sulfonated)	> 100	--	> 100	--
D-8 - Copper stearate	40% kill	--	--	--
D-9 - NCEL No. 838 Copper sulfate and 3-(2'-hydroxy- 5'-nitrophenylazo)-benzene- sulfonic acid, ratio of 1:2	50*	19.4	6.2	25

*Estimated

Table VI. (Cont'd)

D. Organo-Metallic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
D-10 - NCEL No. 839 Copper sulfate and 3-(2'-hydroxy-5'-nitrophenylazo)-benzene-sulfonic acid, ratio of 1:1	45*	19.4	6.2	25
D-11 - NCEL No. 840 Copper sulfate and 3-(2'-hydroxy-5'-nitrophenylazo)-benzene-sulfonic acid, ratio of 2:1	40*	19.4	6.2	25
D-12 - NCEL No. 743 <u>p</u> -Aminophenylmercuric acetate	<1.5	--	<1.5	--
D-13 - NCEL No. 11-430 <u>p</u> -Chloromercuriphenol	<0.1	--	1	--
D-14 - NCEL No. 744 2,6-Diacetoxy-mercuri-gamma-pyrone	80*	--	25	--
D-15 - NCEL No. 727 <u>p</u> -Dimethylaminophenylmercuric acetate	0.05	--	--	--
D-16 - NCEL No. 866 <u>p</u> -Dimethylaminophenylmercuric acetate	0.06	7.8	<1.5	25
D-17 - NCEL No. 938 Diphenyl mercury	8.3	15.1	>100	25

*Estimated

Table VI. (Cont'd)

D. Organo-Metallic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
D-18 - NCEL No. 11-688 Merbromin	1.5	--	25	--
D-19 - NCEL No. 765 Mercurated dichlorophene	13.8	--	25	--
D-20 - NCEL No. 898 Mercurated hydrazobenzene	0.03	9.9	< 1.5	25
D-21 - NCEL No. 778 Mercurated malachite green	52.0	--	< 1.5	--
D-22 - NCEL No. 887 Mercurated β -naphthylamine	6.6	21.1	<12.5	25
D-23 - NCEL No. 1023 Mercurated phenol	14.3	14.6	<12.5	--
D-24 - NCEL No. 750 Mercurated rosaniline	4.0	--	6.2	--
D-25 - NCEL No. 1001 Mercuric pentachlorophenate	>100	--	>100	--
D-26 - NCEL No. 2014 Mercury chelate of kojic acid	0.95	14.7	< 1.5	--
D-27 - NCEL No. 748 Mercurithiosalicylic acid, ethyl ester, sodium salt	0.01	--	< 1.5	--

Table VI. (Cont'd)

D. Organometallic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
D-28 - NCEL No. 752 1-Naphthylmercuric acetate	0.006	--	<1.5	--
D-29 - NCEL No. 11-324 Phenylmercuric chloride	0.5*	--	1	--
D-30 - NCEL No. 11-324 Phenylmercuric chloride	< 1.5	--	--	--
D-31 - NCEL No. 2061 Dibutyltin diacetate	> 100	20.6	< 12.5	--
D-32 - NCEL No. 2062 Dibutyltin dichloride	20.9	20.6	< 12.5	--
D-33 - NCEL No. 2063 Dibutyltin dilaurate	> 100	20.6	< 12.5	--
D-34 - NCEL No. 2059 Dibutyltin maleate	75.1	20.6	< 12.5	--
D-35 - NCEL No. 2064 Dibutyltin oxide	> 100	20.6	< 12.5	--
D-36 - NCEL No. 2137 Tetrabutyltin	104	9.0	--	--
D-37 - NCEL No. 2060-2136 Tetraphenyltin	> 100	20.6	> 100	--

*Estimated

Table VI. (Cont'd)

D. Organo-Metallic Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
D-38 - NCEL No. 994 Tributyltin, coconut fatty acid salt	2.7	5.2	< 3.1	--
D-39 - NCEL No. 993 Tributyltin complex	5.2	5.2	< 3.1	--
D-40 - NCEL No. 2055 Tributyltin oxide	5.6	9.6	< 1.5	--

Table VI. (Cont'd)

E. Surface-Active Agents

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
E-1 - NCEL No. 935 N-Alkyl-N, N-bisdioxyethylene- amine	15.9	15.1	< 1.5	25
E-2 - NCEL No. 929 N-Alkyl-N, N-bispoly- oxyethyleneamine	>100	27.1	6.2	25
E-3 - NCEL No. 936 N-Alkyl-N, N-bispoly- oxyethyleneamine	>100	15.1	< 1.5	25
E-4 - NCEL No. 931 N-Alkyl-N, N-bispoly- oxyethyleneamine	>100	27.1	25	25
E-5 - NCEL No. 1003 Alkyl(C ₁₂ to C ₁₆)dimethyl- benzylammonium chloride	36.8	--	25	--
E-6 - NCEL No. 945 Alkyl(C ₉ to C ₁₈)dimethyl- 3,4-dichlorobenzylammonium bromide	57.3	9.0	25	25
E-7 - NCEL No. 764 Alkyl(C ₉ to C ₁₅)tolymethyl- trimethylammonium chloride	3.6	--	6.2	--
E-8 - NCEL No. 2007 N-Alkyl-trimethylene diamine	8.0	14.7	<6.2	--

Table VI. (Cont'd)

E. Surface-Active Agents

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
E-9 - NCEL No. 2010 N-cocomorpholine	4.8	14.7	< 6.2	100
E-10 - NCEL No. 973 2,1'-Dialkyl-2-alkyl- imidazolinium chloride (av. mol. wt. 370)	90*	--	< 100	--
E-11 - NCEL No. 768 2,1'-Dialkyl-2-alkyl- imidazolinium chloride (av. mol. wt. 450)	41.7	--	100	--
E-12 - NCEL No. 974 2,1'-Dialkyl-2-alkyl- imidazolinium chloride (av. mol. wt. 450)	< 50	--	< 100	--
E-13 - NCEL No. 975 2,1'-Dialkyl-2-alkyl- imidazolinium chloride (av. mol. wt. 455)	>100	--	< 100	--
E-14 - NCEL No. 924 Dialkyldimethylammonium chloride	>100	27.1	>100	50
E-15 - NCEL No. 946 p-(Diisobutyl)cresoxyethoxy- ethyldimethylbenzylammonium chloride	24.5	9.01	6.2	25

*Estimated

Table VI. (Cont'd)

E. Surface-Active Agents

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
E-16 - NCEL No. 928 Dimethylalkyl(C ₁₆ to C ₁₈)amine	2.6	27.1	< 1.5	25
E-17 - NCEL No. 934 Dimethylalkyl(C ₁₂ to C ₁₄)amine	5.2	15.1	< 1.5	25
E-18 - NCEL No. 1005 Dodecylpyridinium chloride	12.4	--	< 12.5	--
E-19 - NCEL No. 927 1-(2-Hydroxyethyl)-2-n-alkyl- 1(or 3)-benzyl-2-imidazolinium chloride (mol. wt. 413)	23.0	27.1	100	25
E-20 - NCEL No. 920 1-(2-Hydroxyethyl)-2-n-alkyl- 1(or 3)-benzyl-2-imidazolinium chloride (mol. wt. 476)	16.6	--	< 1.5	50
E-21 - NCEL No. 921 1-(2-Hydroxyethyl)-2-n-alkyl- 1(or 3)-benzyl-2-imidazolinium chloride (mol. wt. 477)	17.6	11.6	12.5	50
E-22 - NCEL No. 922 1-(2-Hydroxyethyl)-2-n-alkyl- 1-(or 3)-(4-chlorobutyl)-2- imidazolinium chloride (mol. wt. 413)	> 100	11.6	12.5	50

Table VI. (Cont'd)

E. Surface-Active Agents

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
E-23 - NCEL No. 923 1-(2-Hydroxyethyl)-2-n-alkyl- 1-(or 3)-(4-chlorobutyl)-2- imidazolinium chloride (mol. wt. 476)	44.2	11.6	25	50
E-24 - NCEL No. 926 1-(2-Hydroxyethyl)-2-n-alkyl- 1-(or 3)-(4-chlorobutyl)-2- imidazolinium chloride (mol. wt. 477)	>100	27.1	6.2	25
E-25 - NCEL No. 947 Octylphenoxyethoxyethyl, dimethyl, <i>p</i> -chlorobenzyl ammonium chloride	29.8	9.0	25	25
E-26 - NCEL No. 769 Stearyltrimethylbenzyl- ammonium chloride	>100	--	25	--
E-27 - NCEL No. 2011 α -Sulfostearic acid	>100	14.7	>100	--
E-28 - NCEL No. 2008 α -Sulfopalmitic acid	>100	14.7	>100	--
E-29 - NCEL No. 754 tertiary-Octylphenyl hydroxy- ethyl polyether	33.4	--	< 1.5	--

Table VI. (Cont'd)

E. Surface-Active Agents

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
E-30 - NCEL No. 972 Tetramethylammonium chloride	50*	10*	< 100	--
E-31 - NCEL No. 2006 N,N'-Trioxyethylenetri-methylenediamine	29.8	14.7	< 6.2	--

*Estimated

Table VI. (Cont'd)

F. Pesticides

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
F-1 - NCEL No. 877 Benzyl benzoate	>100	--	<12.5	25
F-2 - NCEL No. 2058 2,3,4,5-Bis(2 butylene)tetra- hydrofurfural	>100	14.2	<12.5	--
F-3 - NCEL No. 888-889 2-(<u>p</u> -tertiary-Butylphenoxy)- isopropyl-2-chloroethyl sulfite	>100	--	--	--
F-4 - NCEL No. 832 Chlordane	2.9	12.3	>100	25
F-5 - NCEL No. 853 Chlordane	0.72	9.3	>100	25
F-6 - NCEL No. 2092 Chlordane	6.8	17.4	25	<12.5
F-7 - NCEL No. 854 <u>p</u> -(<u>p</u> -chlorophenoxy)benzene- sulfinic acid	<1.5	9.3	100	25
F-8 - NCEL No. 2096 <u>p</u> -(<u>p</u> -chlorophenoxy)benzene- sulfinic acid	2.8	17.4	100	<12.5

Table VI. (Cont'd)

F. Pesticides

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
F-9 - NCEL No. 758 <u>p</u> -Chlorophenyl phenyl sulfone	>100	--	25	25
F-10 - NCEL No. 999 Cycloheximide	< 25	8.6	>100	<12.5
F-11 - NCEL No. 884 <u>p</u> -Dichlorobenzene	79.0	21.1	>100	25
F-12 - NCEL No. 1004 <u>p</u> -Dichlorobenzene	48.4	--	>100	--
F-13 - NCEL No. 826 Dichlorodiphenyltrichloroethane	< 1.5	11.0	> 100	25
F-14 - NCEL No. 851 Dichlorodiphenyltrichloroethane	< 1.5	9.3	> 100	25
F-15 - NCEL No. 2091 Dichlorodiphenyltrichloroethane	1.3	17.4	> 8	< 12.5
F-16 - NCEL No. 954 Dieldrin	0.29	8.8	> 100	25
F-17 - NCEL No. 2095 Dieldrin	0.71	17.4	> 1.6	< 12.5
F-18 - NCEL No. 930 <i>N,N</i> -Diethyl- <u>m</u> -toluamide	> 100	27.1	> 100	25
F-19 - NCEL No. 11-689 Diphenylaminechlorarsine	25*	--	< 1.5	--

*Estimated

Table VI. (Cont'd)

F. Pesticides

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
F-20 - NCEL No. 939 Diphenyl carbonate	>100	15.1	>100	25
F-21 - NCEL No. 11-563 1,3-Diphenyltriazine	80*	--	25	--
F-22 - NCEL No. 2057 Di-n-propylisocinchomerate	>100	14.2	100	6.2
F-23 - NCEL No. 953 Endrin	<<1.5	8.8	>100	25
F-24 - NCEL No. 1133 Endrin	0.015	8.6	--	--
F-25 - NCEL No. 1136 Endrin	0.111	--	--	--
F-26 - NCEL No. 1137 Endrin	0.032	--	>1	--
F-27 - NCEL No. 2094 Endrin	0.11	17.4	>0.8	<12.5
F-28 - NCEL No. 2056 2-Hydroxyethyl-N-octyl sulfide	32.1	20.6	>100	--
F-29 - NCEL No. 825 Lindane	<1.5	11.0	100	25

*Estimated

Table VI. (Cont'd)

F. Pesticides

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
F-30 - NCEL No. 852 Lindane	0.029	9.3	>100	25
F-31 - NCEL No. 2090 Lindane	<0.25	17.4	2	<12.5
F-32 - NCEL No. 830-850 Methoxychlor	<1.5	10.8	>100	25
F-33 - NCEL No. 2093 Methoxychlor	1.5	17.4	>25	< 12.5
F-34 - NCEL No. 700 2,2'-Methylenebis-4-chloro-phenol	17.1	--	6.2 1.5	--
F-35 - NCEL No. 699 2,2'-Methylenebis-(3,4,6-trichlorophenol)	32.0	--	6.2 1.5	--
F-36 - NCEL No. 1024 N-Methyl-1-naphthylcarbamate	0.012	11.7	< 12.5 > 3.0	6.2
F-37 - NCEL No. 2067 Octachlorocamphene	2.2	20.6	50	6.2
F-38 - NCEL No. 970 Octachlorocamphene	40.2	10*	>100	<12.5
F-39 - NCEL No. 833 Parathion	0.084	12.3	6.2	25

*Estimated

Table VI. (Cont'd)

F. Pesticides

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
F-40 - NCEL No. 777 Pentachlorophenol	24.6	--	6.2	--
F-41 - NCEL No. 958 Pentachlorophenol	72.9	15.9	6.2	25
F-42 - NCEL No. 11-681 Phenothiazene	100*	--	100	--
F-43 - NCEL No. 2003 Pyrethrins-7.5%, piperonyl butoxide-75%, remainder plant extracts	0.46	14.7	3.1	< 12.5

*Estimated

Table VI. (Cont'd)

G. Extractives of Wood and Plants

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
G-1 - NCEL No. I-549 Gallic acid	> 100	--	--	--
G-2 - NCEL No. I-483 Greenheart bark, ether insoluble alkaloids (Ether insoluble fraction of crude basic materials)	> 100	--	> 100	--
G-3 - NCEL No. I-471-477 Greenheart bark, ether soluble alkaloids (Ether soluble fraction of crude basic materials)	>100 > 100	--	> 100 100	--
G-4 - NCEL No. II-440 Greenheart bark, purified alkaloid hydrochlorides from alkaloid E	>100	--	10	--
G-5 - NCEL No. II-461 Greenheart bark, purified alkaloid hydrochlorides from alkaloid F	>100	--	10	--
G-6 - NCEL No. II-464 Greenheart bark, purified alkaloid hydrochlorides from alkaloid G	>100	--	10	--
G-7 - NCEL No. 740 Greenheart bark, chloroform extract of bark previously extracted with acid	>100	--	100	--
G-8 - NCEL No. 836-837 Greenheart, chloroform extract of crude basic materials	>100 > 100	19.4	6.2 6.2	25

Table VI. (Cont'd)

G. Extractives of Wood and Plants

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
G-9 - NCEL No. 741 Greenheart, chloroform extract of sawdust previously extracted with acid	> 100	--	>100	--
G-10 - NCEL No. I-580-610 Greenheart sawdust, alcoholic and acetone extracts	> 100 > 100	--	--	--
G-11 - NCEL No. I-579-576 Greenheart sawdust, aqueous extract	> 100 > 100	--	--	--
G-12 - NCEL No. I-581 Greenheart sawdust, ether extract	> 100	--	--	--
G-13 - NCEL No. I-486 Greenheart sawdust, ether insoluble alkaloids (Ether insoluble fraction of crude basic materials)	> 100	--	> 100	--
G-14 - NCEL No. I-474-480 Greenheart sawdust, ether soluble alkaloids (Ether soluble fraction of crude basic materials)	>100 >100	--	100 100	--
G-15 - NCEL No. 733 Methylumbelliferone	>100	--	--	--
G-16 - NCEL No. I-592 Morin	>100	--	--	--

Table VI. (Cont'd)

G. Extractives of Wood and Plants

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
G-17 - NCEL No. 2065 Octca rubra, crude wax extracted	> 100	20.6	< 12.5	--
G-18 - NCEL No. 806 Pyrogallol	> 100	8.68	> 100	25
G-19 - NCEL No. II-99 Quinine	> 100	--	--	--
G-20 - NCEL No. 583 Redwood extracts, alcoholic	> 100	--	--	--
G-21 - NCEL No. 751 Redwood extracts, alcoholic	> 100	--	> 100	--
G-22 - NCEL No. 582 Redwood extracts, aqueous	> 100	--	--	--
G-23 - NCEL No. 779 Redwood extracts, chloroform	> 100	--	100	--
G-24 - NCEL No. I-584 Redwood extracts, ether	> 100	--	--	--
G-25 - NCEL No. 812 Redwood, steam distillate	> 100 25*	8.7	100	25
G-26 - NCEL No. I-602 Rutin	> 100	--	--	--

*Estimated

Table VI. (Cont'd)

G. Extractives of Wood and Plants

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
G-27 - NCEL No. 702 Strychnidine	> 100	--	25	--
G-28 - NCEL No. 811 Tannic acid	> 100	8.7	> 100	25
G-29 - NCEL No. 841 Vanillin	> 100	19.4	> 100	25

Table VI. (Cont'd)

H. Haloacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
H-1 - NCEL No. 995 Bromoacetic acid	4.3	8.6	6.2	50
H-2 - NCEL No. 1083 Bromoacetic acid	3.9	14.2	<1.5	12.5
H-3 - NCEL No. 900 Bromoacetic acid, benzyl ester	6.2	7.7	<0.3	50
H-4 - NCEL No. 899 Bromoacetic acid, 1,3-butane-diol diester	4.5	7.7	<1.5 <0.3	50
H-5 - NCEL No. 904 Bromoacetic acid, 2-butyloctyl ester	65.5	7.7	6.2	50
H-6 - NCEL No. 903 Bromoacetic acid, 2-chloroethyl ester	5.4	7.7	<1.5 <0.3	50
H-7 - NCEL No. 907 Bromoacetic acid, <i>p</i> -chlorophenyl-ethyl ester	7.9	7.7	25 3	50
H-8 - NCEL No. 896 Bromoacetic acid, 2-cyclohexyl-ethyl ester	5.1	7.7	1.5 3	50

Table VI. (Cont'd)

H. Haloacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
H-9 - NCEL No. 906 Bromoacetic acid, <u>n</u> -dodecyl ester	35.2	7.7	25 > 3	50
H-10 - NCEL No. 901 Bromoacetic acid, ethylene glycol diester	5.9	7.7	<1.5 <0.3	50
H-11 - NCEL No. 914 Bromoacetic acid, <u>n</u> -hexyl ester	4.3	7.7	<1.5 <0.3	50
H-12 - NCEL No. 905 Bromoacetic acid, 2-(2-methoxy- ethoxy)ethyl ester	7.4	7.7	6.2 3	50
H-13 - NCEL No. 912 Bromoacetic acid, 2-methoxyethyl ester	3.4	7.7	<1.5 3	50
H-14 - NCEL No. 911 Bromoacetic acid, <u>p</u> -nitrobenzyl ester	53.0	7.7	<1.5 > 3	50
H-15 - NCEL No. 902 Bromoacetic acid, <u>n</u> -octyl ester	3.3	7.7	<1.5 3	50
H-16 - NCEL No. 932 Bromoacetic acid, <u>n</u> -octyl ester	5.0	27.1	<3.1 1.0	25
H-17 - NCEL No. 1070 Bromoacetic acid, <u>n</u> -octyl ester	8.1	14.2	<1.5 3	--

Table VI. (Cont'd)

H. Haloacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Cresote	Agent	Cresote
H-18 - NCEL No. 910 Bromoacetic acid, 1,5-pentanediol diester	4.3	7.7	<1.5 3	--
H-19 - NCEL No. 915 Bromoacetic acid, 2-phenethyl ester	7.0	7.7	<1.5	--
H-20 - NCEL No. 913 Bromoacetic acid, 1,2-propanediol diester	26.3	7.7	<1.5 > 3	--
H-21 - NCEL No. 909 Bromoacetic acid, 2,2,2-trichloroethyl ester	6.3	7.7	<1.5 3	--
H-22 - NCEL No. 1082 Chloroacetic acid	3.7	14.2	>50	--
H-23 - NCEL No. 1064 Chloroacetic acid, 4-biphenyl ester	6.9	14.2	12.5 6.2	--
H-24 - NCEL No. 1069 Chloroacetic acid, <u>n</u> -(2-butyl)-octyl ester	2.6	14.2	50 > 6.2	--
H-25 - NCEL No. 1078 Chloroacetic acid, <u>p</u> -tertiary-butyl- <u>o</u> -tolyl ester	12.5	14.2	12.5 > 6.2	12.5

Table VI. (Cont'd)

H. Haloacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
H-26 - NCEL No. 1072 Chloroacetic acid, <u>o</u> -chlorophenyl ester	10.7	14.2	12.5 > 6.2	12.5
H-27 - NCEL No. 1073 Chloroacetic acid, <u>p</u> -chlorophenyl ester	9.3	14.2	50 > 6.2	12.5
H-28 - NCEL No. 1075 Chloroacetic acid, <u>p</u> -chloro- <u>o</u> -tolyl ester	10.4	14.2	12.5 25	--
H-29 - NCEL No. 1079 Chloroacetic acid, <u>o</u> -chloro- <u>o</u> -tolyl ester	--	14.2	12.5 > 6.2	12.5
H-30 - NCEL No. 1074 Chloroacetic acid, 2,4-dichloro-phenyl ester	13.8	14.2	12.5 6.2	12.5
H-31 - NCEL No. 1067 Chloroacetic acid, isobutyl ester	9.1	14.2	12.5 6.2	12.5
H-32 - NCEL No. 1065 Chloroacetic acid, isopropyl ester	7.5	14.2	12.5 6.2	12.5
H-33 - NCEL No. 1077 Chloroacetic acid, <u>o</u> -nitrophenyl ester	9.6	14.2	> 50 > 6.2	12.5

Table VI. (Cont'd)

H. Halocacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
H-34 - NCEL No. 1080 Chloroacetic acid, <i>p</i> -nitrophenyl ester	12.7	14.2	> 50 > 25	12.5
H-35 - NCEL No. 1068 Chloroacetic acid, 2- <i>octyl</i> ester	12.8	14.2	12.5 6.2	12.5
H-36 - NCEL No. 1066 Chloroacetic acid, 3-phenyl- <i>propyl</i> ester	8.5	14.2	12.5 6.2	12.5
H-37 - NCEL No. 1081 Chloroacetic acid, 2,3,4,6-tetrachlorophenyl ester	29.1	14.2	12.5 > 6.2	12.5
H-38 - NCEL No. 1071 Chloroacetic acid, <i>o</i> -tolyl ester	9.1	14.2	50 > 6.2	12.5
H-39 - NCEL No. 1076 Chloroacetic acid, 2,4,5-trichlorophenyl ester	15.4	14.2	12.5 6.2	12.5
H-40 - NCEL No. 1084 Fluoroacetic acid	> 100	14.2	> 50	25
H-41 - NCEL No. II-542 Fluoroacetic acid	> 100	--	> 100	--
H-42 - NCEL No. III-4 Iodoacetic acid and its sodium salt	6.0	--	--	--

Table VI. (Cont'd)

H. Haloacetic Acids and Their Derivatives

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
H-43 - NCEL No. II-391 Iodoacetic acid and its sodium salt	6.2	--	<1	--
H-44 - NCEL No. 1085 Iodoacetic acid and its sodium salt	7.0	14.2	3.1	--
H-45 - NCEL No. 703 Iodoacetocetic acid, ethyl ester	--	--	25	--
H-46 - NCEL No. 701 Iodoacetone	19.8	--	100	--

Table VI. (Cont'd)

I. Other Aliphatics

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
I-1 - Acetone	> 100	--	>100	25
I-2 - NCEL No. I-327 Acetylcholine chloride	> 100	--	>100	--
I-3 - NCEL No. 863 Aconitic acid	> 100	12.0	>100	25
I-4 - NCEL No. II-685 Acrylonitrile	50*	--	>100	--
I-5 - NCEL No. II-686 Adiponitrile	> 100	--	>100	--
I-6 - NCEL No. 940 Allylglycine	> 100	15.1	25	25
I-7 - NCEL No. 714 Butyne-1,4-Diol	> 100	--	>100	--
I-8 - NCEL No. II-84 Cellosolve	> 100	--	>100	--
I-9 - NCEL No. 894 Chlorinated paraffin	> 100	9.9	>100	25
I-10 - NCEL No. I-545 Choline chloride	>10	--	>100	--

*Estimated

Table VI. (Cont'd)

I. Other Aliphatics

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
I-11 - NCEL No. II-697 N,N-Diethyl-1,3-propanediamine	> 100	--	>100	--
I-12 - NCEL No. 787 1,1-Dimethylhydrazine	> 100	12.6	100	--
I-13 - NCEL No. 732 Dithiocarbamylpropionic acid	100*	--	--	--
I-14 - Ethanol	> 1%	--	>1%	--
I-15 - NCEL No. 1002 D,L-Ethionine	100*	--	<12.5	--
I-16 - Ethyl acetate	< 1%	--	--	--
I-17 - NCEL No. 847 Ethylene glycol	< 100	11.7	>100	--
I-18 - NCEL No. II-530 Formaldehyde	50*	--	10	--
I-19 - NCEL No. 1047 Formamide	>1%	18.4	>1%	--
I-20 - NCEL No. II-539 Formic acid	> 100	--	100	--

*Estimated

Table VI. (Cont'd)

I. Other Aliphatics

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
I-21 - NCEL No. II-189 Glycine	> 1000	--	--	--
I-22 - NCEL No. II-696 1,6-Hexanediamine	>100	--	>100	--
I-23 - NCEL No. 734 β -Isothioureido propionic acid	>100	--	--	--
I-24 - NCEL No. 722 Lanthionine (L + meso)	>100	--	--	--
I-25 - NCEL No. 738 Lanthionine sulfone	>100	--	>100	--
I-26 - NCEL No. 2002 Malonic acid	>100	14.7	>100	--
I-27 - NCEL No. 728 N-Methylglucamine	>100	--	--	--
I-28 - NCEL No. 844 O-Methylhydroxylamine hydrochloride	5*	11.7	>100	25
I-29 - NCEL No. 755 ω -Methylpantothenic acid, calcium salt	--	--	6.25	--
I-30 - NCEL No. 807 Oxalic acid	>100	8.7	>100	25

*Estimated

Table VI. (Cont'd)

I. Other Aliphatics

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
I-31 - NCEL No. 876 <u>d</u> -Pantoyltaurine	>500	--	> 500	25
I-32 - NCEL No. II-533 Paraformaldehyde	50*	--	100	--
I-33 - NCEL No. 2080 Petroleum Oil #30	>100	14.7	> 100	--
I-34 - NCEL No. 2005 Polyvinylacetate, modified resin, solution in dilute alkalies	>100	14.7	> 100	--
I-35 - NCEL No. 759 Polyvinylmethyl ether, maleic anhydride copolymer	>100	--	> 100	--
I-36 - NCEL No. II-695 Putrescine dihydrochloride	>100	--	> 100	--
I-37 - NCEL No. 829 Trichloroethane sulfenyi chloride	>100	12.3	> 100	25
I-38 - NCEL No. 2001 Trimethylacetic acid	>100	14.7	> 100	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-1 - NCEL No. I-661 5-(p-Acetamidophenylazo)-8-quinolinol hydrochloride	>100	--	>20	--
J-2 - NCEL No. I-636 p-(4-Amino-1-naphthylazo)-benzenesulfonic acid, sodium salt	>100	--	>20	--
J-3 - NCEL No. I-626 p-(p-Aminophenylazo)benzenesulfonic acid	>100	--	--	--
J-4 - NCEL No. I-638 3-(4-Anilino-1-naphthylazo)-2,7-naphthalenedisulfonic acid	100*	--	>20	--
J-5 - NCEL No. I-627 m-(p-Anilinophenylazo)benzenesulfonic acid	>100	--	>20	--
J-6 - NCEL No. II-628 p-(p-Anilinophenylazo)benzenesulfonic acid	>100	--	100	--
J-7 - NCEL No. I-556 Azobenzene	0.42	--	--	--
J-8 - NCEL No. II-556 Azobenzene	1.4 2.6	--	25	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-9 - NCEL No. I-554 Azoxybenzene	1.5	--	--	--
J-10 - NCEL No. II-554 Azoxybenzene	7.5 2.7	--	25	--
J-11 - NCEL No. 810 Bismarck brown	>100	8.7	25	25
J-12 - NCEL No. II-662 1,3-Bis(<u>p</u> -nitrophenyl)triazine	>100	--	--	--
J-13 - NCEL No. I-654 Bis(phenylazo)resorcinol	>100	--	>20	--
J-14 - NCEL No. I-577 Brilliant yellow	>100	--	>2	--
J-15 - NCEL No. I-643 Brilliant vital red	>100	--	>20	--
J-16 - NCEL No. I-644 Clayton yellow	>100	--	>20	--
J-17 - NCEL No. II-690, 861 Congo red	>100 >100	12.0	>100 >100	25
J-18 - NCEL No. I-665 Diethyl- <u>p</u> , <u>p</u> '-azodibenzoate	>100	--	--	--

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-19 - NCEL No. I-639 4,5-Dihydroxy-3-(<i>p</i> -nitrophenylazo)-2,7-naphthalenedisulfonic acid, disodium salt	>100	--	> 20	--
J-20 - NCEL No. I-632 <i>p</i> -(2,4-Dihydroxyphenylazo)-benzenesulfonic acid, sodium salt	>100	--	> 20	--
J-21 - NCEL No. I-664 N,N-Dimethyl- <i>p</i> -1-naphthylazo-aniline	>100	--	--	--
J-22 - NCEL No. I-663 N,N-Dimethyl- <i>p</i> -2-naphthyl-azoaniline	>100	--	--	--
J-23 - NCEL No. I-647 N,N-Dimethyl- <i>p</i> -phenylazo-aniline	>100	--	>100	--
J-24 - NCEL No. II-648 4-(3,5-Dimethyl-phenylazo)-3,5-dimethylaniline hydrochloride	>100	--	25	--
J-25 - NCEL No. 885 Hydrazobenzene	4*	21.1	>100	25
J-26 - NCEL No. 895 Hydrazobenzene	3.8	9.9	50	25

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-27 - NCEL No. I-670 3-(4-Hydroxy-3-methylphenylazo)-benzenesulfonic acid	>100	--	--	--
J-28 - NCEL No. I-666,674 3-(4-Hydroxy-2-methylphenylazo)-benzenesulfonic acid	100*	--	--	--
J-29 - NCEL No. I-668 3-(2-Hydroxy-5-methylphenylazo)-benzenesulfonic acid	>100	--	--	--
J-30 - NCEL No. I-637 p-(2-Hydroxy-1-naphthylazo)-benzenesulfonic acid, sodium salt	>100	--	--	--
J-31 - NCEL No. I-634 1-(2-Hydroxy-1-naphthylazo)-2-naphthol-4-sulfonic acid, zinc salt	100*	--	--	--
J-32 - NCEL No. I-633 1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid, sodium salt	>100	--	--	--
J-33 - NCEL No. I-672 3-(2-Hydroxy-5'-nitrophenylazo)-benzenesulfonic acid and its sodium salt	25*	--	< 1.5	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-34 - NCEL No. I-667,669 <u>m</u> -(p-Hydroxyphenylazo)benzene-sulfonic acid and its sodium salt	> 100 > 100	--	--	--
J-35 - NCEL No. I-629 <u>p</u> -(p-Hydroxyphenylazo)benzene-sulfonic acid and its sodium salt	> 100	--	--	--
J-36 - NCEL No. I-659 4-(8-Hydroxy-5-quinolylazo)-1-naphthalenesulfonic acid	> 100	--	--	--
J-37 - NCEL No. I-501 <u>p</u> -Methoxyazobenzene	< 1.0	--	--	--
J-38 - NCEL No. II-501 <u>p</u> -Methoxyazobenzene	0.27	--	>100	--
J-39 - NCEL No. II-498 <u>p</u> -Methoxyazoxybenzene	8*	--	--	--
J-40 - NCEL No. I-649 2-Methoxy-4-(<u>o</u> -methoxyphenyl-azo)aniline	> 100	--	--	--
J-41 - NCEL No. I-640 Methyl-1,4-phenylazophenol	4.5*	--	--	--
J-42 - NCEL No. II-640 3-Methyl-1,4-phenylazophenol	25*	--	6.2	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-43 - NCEL No. II-673 <u>m</u> -Nitroazobenzene	> 100	--	6.2	--
J-44 - NCEL No. I-658 4-(<u>p</u> -Nitrophenylazo)-1-naphthol	> 100	--	--	--
J-45 - NCEL No. I-653 4-(<u>p</u> -Nitrophenylazo)orcinol	> 100	--	--	--
J-46 - NCEL No. II-671 <u>p</u> -(<u>p</u> -Nitrophenylazo)phenol	12*	--	< 1.5	--
J-47 - NCEL No. II-650 4-(<u>m</u> -Nitrophenylazo)resorcinol	> 100	--	6.2	--
J-48 - NCEL No. II-655 4-(<u>p</u> -Nitrophenylazo)resorcinol	> 100	--	6.2	--
J-49 - NCEL No. I-641 5-(<u>p</u> -Nitrophenylazo)salicylic acid, sodium salt	> 100	--	--	--
J-50 - NCEL No. II-518 <u>p</u> -Phenylazoaniline	3*	--	> 100	--
J-51 - NCEL No. I-631 <u>p</u> -Phenylazobenzenesulfonic acid	> 100	--	--	--
J-52 - NCEL No. I-507 <u>m</u> -Phenylazobenzoic acid	50*	--	> 100	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-53 - NCEL No. II-507 m-Phenylazobenzoic acid	10*	--	--	--
J-54 - NCEL No. II-660 p-Phenylazobenzoic acid	>100	--	>100	--
J-55 - NCEL No. II-651 4-Phenylazodiphenylamine	>100	--	>100	--
J-56 - NCEL No. 771 4-Phenylazodiphenylamine	>100	--	--	--
J-57 - NCEL No. II-652 4-Phenylazo-1-naphthylamine	>100	--	>100	--
J-58 - NCEL No. II-645 p-Phenylazophenol	12*	--	25	--
J-59 - NCEL No. I-657 4-Phenylazoresorcinol	9.17	--	--	--
J-60 - NCEL No. II-657 4-Phenylazoresorcinol	24.9	--	25	--
J-61 - NCEL No. II-521 p-Phenylazoxyaniline	>100	--	>100	--
J-62 - NCEL No. II-403 p-Phenylazoxybenzenesulfonic acid	100*	--	>100	--

*Estimated

Table VI. (Cont'd)

J. Organic Azo Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
J-63 - NCEL No. II-400 <u>m</u> -Phenylazoxybenzoic acid	100*	--	>100	--
J-64 - NCEL No. I-559 Sulfo- <u>o</u> -methoxybenzeneazodi- methyl-2-naphthylamine	50*	--	--	--

*Estimated

Table VI. (Cont'd)

K. Dyes and Stains

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
K-1 - NCEL No. 760 Acid fuchsin	>100	--	> 100	
K-2 - NCEL No. II-679 Alizarin	>100	--	> 100	--
K-3 - NCEL No. II-691 Auramine G	>100	--	6.2	--
K-4 - NCEL No. I-605 Aurin	>100	--	25	--
K-5 - NCEL No. 774 Benzopurpurin	>100	--	--	--
K-6 - NCEL No. II-677 Brilliant green	>100	--	< 1.5	--
K-7 - NCEL No. 858 Bromcresol green	>100	12.0	> 100	25
K-8 - NCEL No. 775 <u>o</u> -Cresolphthalein	>100	--	--	--
K-9 - NCEL No. II-553 Crystal violet	12*	--	< 1.5	--
K-10 - NCEL No. 803 Erythrosine	>100	18.2	25	25

*Estimated

Table VI. (Cont'd)

K. Dyes and Stains

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
K-11 - NCEL No. 849 Hydroxy malachite green	> 100	9.29	25	25
K-12 - NCEL No. 737 Indigo carmine	> 100	--	>100	--
K-13 - NCEL No. 862 Lacmoid	> 100	12.0	> 100	25
K-14 - NCEL No. II-552 Malachite green, oxalate	> 100	--	< 1.5	--
K-15 - NCEL No. 868 Methylene blue	> 100	7.81	25	25
K-16 - NCEL No. II-676 Methyl green	> 100	--	25	--
K-17 - NCEL No. II-675 Methyl violet	100	--	< 1.5	--
K-18 - NCEL No. 860 Propyl red	100	12.0	25	25
K-19 - NCEL No. 797 Pyronin, a basic dye	100	14.7	< 1.5	25
K-20 - NCEL No. 1031 to 37 Reactive dyes (A new class of dyes which react chemically with cotton. Several tested)	100	18.4	< 100	--

Table VI. (Cont'd)

K. Dyes and Stains

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
K-21 - NCEL No. 859 Resazurin	100	12.0	>100	25
K-22 - NCEL No. 11-698 Rhodamine B	100	--	100	--
K-23 - NCEL No. 749 Rosaniline hydrochloride	--	--	<1.5	--
K-24 - NCEL No. 871 Safranine-O	>100	7.81	<1.5	25
K-25 - NCEL No. 11-536 Schiff base	50*	--	>100	--
K-26 - NCEL No. 11-678 Spirit blue	>100	--	>100	--
K-27 - NCEL No. 796 Thionin	>100	14.7	<1.5	--
K-28 - NCEL No. 761 Wright's stain	>100	--	>100	--

*Estimated

Table VI. (Cont'd)

L. Aromatic Nitro Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
L-1 - NCEL No. 2012 N-Methyl-N-nitroso- <u>p</u> -toluene- sulfonamide	19.3	14.7	25	--
L-2 - NCEL No. 960 Dinitro- <u>o</u> -cyclohexylphenol	1.34	15.9	< 1.5	25
L-3 - NCEL No. III-1,2 2,4-Dinitrophenol	4.8	--	--	--
L-4 - NCEL No. III-3 2,4-Dinitrophenol	11.2	--	--	--
L-5 - NCEL No. II-512 N-(2,4-Dinitrophenyl)glycine, syn. from 1-chloro-2-4-dinitro- benzene	10*	--	100	--
L-6 - NCEL No. II-515 N-(2,4-Dinitrophenyl)glycine, syn. from 1-fluoro-2,4-dinitro- benzene	50*	--	>100	--
L-7 - NCEL No. 773 Dinitrophenylhydrazine	>100	--	--	--
L-8 - NCEL No. II-524 Di(<u>p</u> -nitrophenyl)urea	>100	--	>100	--
L-9 - NCEL No. II-527 Di(<u>m</u> -nitrophenyl)urea	100	--	>100	--

*Estimated

Table VI. (Cont'd)

L. Aromatic Nitro Compounds

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
L-10 - NCEL No. 956 Dinitro- <u>o</u> - <u>sec</u> -butylphenol	1.9	15.9	<1.5	25
L-11 - NCEL No. 957 Dinitro- <u>o</u> - <u>sec</u> -butylphenol	3.0	15.9	6.25	25
L-12 - NCEL No. 770 <u>o</u> -Nitrobenzenesulfenyl chloride	>100	--	100	--

Table VI. (Cont'd)

M. Quinones

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
M-1 - NCEL No. 968 1-Aminoanthraquinone	>100	15.9	>100	--
M-2 - NCEL No. 967 2-Aminoanthraquinone	>100	15.9	>100	--
M-3 - NCEL No. I-557 9, 10-Anthraquinone	>100	--	--	--
M-4 - NCEL No. II-683 2, 5-Diethanolamine-1, 4-benzoquinone	>100	--	>100	--
M-5 - NCEL No. 705 2, 6-Diiodohydroquinone	>100	--	>100	--
M-6 - NCEL No. 704 Diiodoquinone	>100	--	>100	--
M-7 - NCEL No. 781 2, 6-Dimethylbenzoquinone	>100	--	2.5	--
M-8 - NCEL No. II-692 2, 5-Dimethyl- <i>p</i> -quinone	2.7	--	<1.5	--
M-9 - NCEL No. II-684 2, 5-Dimorpholinyl-1, 4-benzoquinone	>100	--	>100	--
M-10 - NCEL No. II-680 Duroquinone	25*	--	100	--

*Estimated

Table VI. (Cont'd)

M. Quinones

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
M-11 - NCEL No. II-551 2-Methyl-1,4-naphthoquinone	3*	--	25	--
M-12 - NCEL No. 881 1,2-Naphthoquinone	51.2	21.1	<12.5	25
M-13 - NCEL No. II-550 1,4-Naphthoquinone	>100	--	1.5	--
M-14 - NCEL No. I-606 Phenanthraquinone	>100	--	6.2	--
M-15 - NCEL No. I-586 Quinone	2.5	--	6.2	--
M-16 - NCEL No. II-571 Tetrachloroquinone	>100	--	<1.5	--

*Estimated

Table VI. (Cont'd)

N. Aromatic Amines and Phenols

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
N-1 - NCEL No. 706 2-Aminofluorene	1.2	--	> 100	--
N-2 - NCEL No. 729 2-Aminofluorene	1.8	--	> 100	--
N-3 - NCEL No. 919 <u>p</u> -Aminohippuric acid	> 100	11.6	> 100	--
N-4 - NCEL No. 950 5-Amino-1-naphthol	100*	9.0	> 100	25
N-5 - NCEL No. I-608 <u>p</u> -Aminophenol	60*	--	> 100	--
N-6 - NCEL No. II-694 <u>p</u> -Anilinophenol 8	10.4	--	6.2	--
N-7 - NCEL No. I-603 <u>p</u> -Anisidine	> 100	--	> 100	--
N-8 - NCEL No. 709 Benzidine	50*	--	100	--
N-9 - NCEL No. I-574 N-Benzyl-2-hydroxyacetanilide	100*	--	--	--
N-10 - NCEL No. 808 Catechol	80*	8.7	> 100	25

*Estimated

Table VI. (Cont'd)

N. Aromatic Amines and Phenols

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
N-11 - NCEL No. 2103 Catechol	80*	15.3	100	--
N-12 - NCEL No. 998 2-Chloro-4-aminobenzoic acid	25*	8.6	>100	--
N-13 - NCEL No. II-682 <u>m</u> -Chlorophenol	15*	--	100	--
N-14 - NCEL No. I-598 <u>p</u> -Chlorophenol	12*	--	100	--
N-15 - NCEL No. I-599 <u>o</u> -Chlorophenol	10*	--	>100	--
N-16 - NCEL No. 1026 5,7-Dichloro-8-hydroxyquinoline	> 25	14.6	25	< 6.2
N-17 - NCEL No. 711 N-N'-Di-(4-hydroxybenzylidene)- <u>p</u> -phenylenediamine	>100	--	>100	--
N-18 - NCEL No. II-492 3,5-Diodotyrosine	>100	--	>100	--
N-19 - NCEL No. 713 N-N'-Disalicylidene- <u>p</u> -phenyl- enediamine	>100	--	>100	--
N-20 - NCEL No. 955 2,6-Di- <u>tertiary</u> -butyl- <u>p</u> -cresol	100*	8.8	>100	--

*Estimated

Table VI. (Cont'd)

N. Aromatic Amines and Phenols

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
N-21 - NCEL No. I-591 3-Hydroxyflavone	> 100	--	--	--
N-22 - NCEL No. I-72 8-Hydroxyquinoline	100*	--	--	--
N-23 - NCEL No. II-607 Metanilic acid	> 100	--	> 100	--
N-24 - NCEL No. 882 α -Naphthol benzene	> 100	21.1	50	--
N-25 - NCEL No. 780 Pentabromophenol	21.3	12.6	< 1.5	100
N-26 - NCEL No. 782 5-n-Pentadecylresorcinol	> 100	12.6	> 100	--
N-27 - NCEL No. I-566 α -Phenylenediamine	> 100	--	--	--
N-28 - NCEL No. 870 m -Phenylenediamine dihydrochloride	> 100	7.8	> 100	25
N-29 - NCEL No. I-568 p -Phenylenediamine	50*	--	--	--
N-30 - NCEL No. 834 Resorcinol	> 100	12.3	> 100	25

*Estimated

Table VI. (Cont'd)

N. Aromatic Amines and Phenols

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
N-31 - NCEL No. 893 Sulfanilamide	>100	9.8	>100	25
N-32 - NCEL No. 809 2,4,6-Trichlorophenol	26.3	8.7	100	25

Table VI. (Cont'd)

O. Miscellaneous Organic Compounds and Mixtures

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
O-1 - NCEL No. 880 Acetanilide	> 100	21.1	>100	25
O-2 - NCEL No. 891 Allantoin	> 100	9.9	>100	25
O-3 - NCEL No. 918 Benzimidazole	100*	11.6	>100	--
O-4 - NCEL No. I-572 Benzylbenzoxazolium chloride	50*	--	--	--
O-5 - NCEL No. I-573 4-Benzyl-3-diethylaminomethyl- 2,3-dihydro-1,4-benzoxazine dihydrochloride	50*	--	--	--
O-6 - NCEL No. 747 β -Carbonaphthoxycholine iodide	50*	--	25	--
O-7 - NCEL No. 762 $(\beta$ -Carboxyethyl)benzothiazole- 2-sulfide	52.9	--	<1.5	--
O-8 - NCEL No. I-570 Chalcone	5*	--	25	--
O-9 - NCEL No. 867 Chlorinated biphenyl	67.5	7.8	> 100	--

*Estimated

Table VI. (Cont'd)

O. Miscellaneous Organic Compounds and Mixtures

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
O-10 - NCEL No. 2105 Chlorokojic acid	58.5	15.3	25	--
O-11 - NCEL No. 1040 Chlorokojic acid	40*	18.4	50	--
O-12 - NCEL No. 864 Cinnamic acid, (trans),(synthetic)	>100	12.0	>100	--
O-13 - NCEL No. I-569 Coumarin	5*	--	100	--
O-14 - NCEL No. 712 N,N'-Dianisylidene-p-phenyl- enediamine	>100	--	>100	--
O-15 - NCEL No. 708 N,N-dibenzylidenephenylene- diamine	>100	--	>100	--
O-16 - NCEL No. 2000 Dibenzylphthalate	>100	14.7	<100	--
O-17 - NCEL No. 707 N,N'-Di(4-chlorobenzylidene)- phenylenediamine	>100	--	>100	--
O-18 - NCEL No. I-558 5,5-Diethylbarbituric acid, sodium salt	100*	--	--	--

*Estimated

Table VI. (Cont'd)

O. Miscellaneous Organic Compounds and Mixtures

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
O-19 - NCEL No. 883 sym-Diphenylcarbazide	>100	21.1	> 100	25
O-20 - NCEL No. 937 Diphenylphthalate	> 100	15.1	> 100	25
O-21 - NCEL No. 2004 1,3-Diphenyl-2-propanone	29.0	14.7	< 25	12.5
O-22 - NCEL No. 721 Ephedrine sulfate	>100	--	--	--
O-23 - NCEL No. I-548 Histamine	>100	--	--	--
O-24 - NCEL No. 746 Indole-3-acetic acid	>100	--	100	--
O-25 - NCEL No. 2102 Indoxyl acetate	15.4	15.3	<12.5	--
O-26 - NCEL No. 823 Indoxyl acetate	50*	11.0	> 100	25
O-27 - NCEL No. 735 Indoxyl acetate	11.8	--	> 100	--
O-28 - NCEL No. 1041 Kojic acid	> 100	18.4	> 100	--

*Estimated

Table VI. (Cont'd)

O. Miscellaneous Organic Compounds and Mixtures

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
O-29 - NCEL No. 776 Melamine	>100	--	>100	--
O-30 - NCEL No. 917 N-Methyl-N-1-naphthylacetamide	57.0	11.6	>100	50
O-31 - NCEL No. 1016 N-Methyl-N-1-naphthylacetamide	63.6	--	100	12.5
O-32 - NCEL No. 1038 N-2-Naphthyl palmitamide	>100	18.4	25	--
O-33 - NCEL No. 765 Ninhydrin	13.8	--	>100	--
O-34 - NCEL No. 879 Nutrient broth	>100	21.1	>100	25
O-35 - NCEL No. 784 Pentabromophenyl- β -D-glucoside	>100	12.6	25	--
O-36 - NCEL No. 878 Pentachlorobenzenethiol	104.6	--	<12.5	25
O-37 - NCEL No. 786 2,3,4,5,6-Pentachloro-4-penta- chlorophenoxy-2,5-cyclohexa- dienone	46.3	12.6	<1.5	--
O-38 - NCEL No. 785 Pentachlorophenyl- β -D-glucoside	28.6	12.6	6.2	--

Table VI. (Cont'd)

O. Miscellaneous Organic Compounds and Mixtures

CHEMICAL AGENT	LIMNORIA		TEREDO LARVAE	
	Concentration to Kill 50% in 100 hrs		Minimum Concentration to Kill 100% in 72 hrs	
	Agent	Creosote	Agent	Creosote
O-39 - NCEL No. 892 N-Phenylglycine	>100	9.9	>100	25
O-40 - NCEL No. 11-693 Phenylmercaptoacetic acid	>100	--	>100	--
O-41 - NCEL No. 1-547 Procaine hydrochloride	>100	--	--	--
O-42 - NCEL No. 1-555 Quinolinic acid	>100	--	--	--
O-43 - NCEL No. 2009 Tetrabromophthalic anhydride	>100	14.7	>100	25
O-44 - NCEL No. 1044 Tetrachlorophthalic anhydride	6.2	18.4	>100	12.5
O-45 - NCEL No. 788 Tetramethyltetrazine	>100	12.7	>100	--
O-46 - NCEL No. 846 2,4,5-Trichlorophenoxyacetic acid	>100	11.6	>100	25
O-47 - NCEL No. 822 Trichlorophenyl- β -D-glucoside	80*	11.0	>100	25
O-48 - NCEL No. 745 D-L-Tryptophane	>100	--	25	12.5

*Estimated

APPENDIX C

INDEX OF CHEMICALS FOR WHICH TOXICITY DATA ARE AVAILABLE

It is believed that this index lists essentially all the compounds and chemicals for which data concerning toxicity to marine borers has been published. It also lists the compounds which were tested at this laboratory prior to 1959.

INDEX GUIDE

"Ref." followed by a number (e.g., Ref. 10) indicates that information concerning the toxicity of the compound may be found in the bibliography reference of the same number. A capital letter followed by a dash and a number (e.g., B-1) indicates that information on the toxicity of the compound may be found at that location in Table VI of Appendix B.

An asterisk indicates that the name given for the compound at the location cited will be a synonym.

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